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TOXICITY OF GALACTOSE FOR CERTAIN OF THE HIGHER PLANTS[1]

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In the course of investigations upon the effect of sugars on the growth of certain higher plants, the sugar galactose was employed. In experiments with vetch (*Vicia villosa*) the plants grown in the presence of 2 per cent galactose showed very marked injury, the injury being especially manifest by a killing of the roots and accompanied by a reduction in the growth of tops. The results secured were the more surprising in view of the fact that lactose sugar employed coincidently influenced beneficially the growth of the same plant. Certain experiments were therefore made to determine whether or not the effect of the galactose was consistent.

Method of experimentation.—The plants were grown under sterile conditions on agar media containing Pfeffer's nutrient solution[2] of one-half its normal strength. This solution is neutral in its reaction. The solution contained varying amounts of galactose sugar, the source of which is indicated in each case.

[1] The writer acknowledges gladly his indebtedness to the officers of the Missouri Botanical Garden for facilities and courtesies extended to him during his stay in St. Louis.

[2] $CaNO_3$ ..... 2 grams
KNO_3 ..... 0.5 grams
KCl ..... 0.25 grams
K_2HPO_4 ..... 0.50 grams
$MgSO_4$ ..... 0.50 grams
Fe_2Cl_3 ..... 4 milligrams
Dist. water ..... 6 liters

The seed employed were sterilized by means of a method devised in the Laboratory of Plant Physiology of Cornell University by Dr. J. K. Wilson.[1] In brief it is as follows: 10 grams of chloride of lime are shaken up with 150 cc. tap water and after standing for ten minutes the supernatant liquid is filtered. The filtrate is used as the sterilizing agent. The seeds are placed in a test-tube covered with about five times their volume of the filtrate and the tube then tightly stoppered. The seeds are treated for from 4 to 24 hours, depending upon the character of the seed. In the experiments here mentioned the vetch seeds were exposed to this treatment for 12 hours and the peas for 4 hours. The seeds are directly transferred to the culture vessels from the chloride of lime solution, care being observed to drain off all of the chloride of lime solution. In transferring the seed the usual bacteriological precautions are observed.

Experiment with vetch (*Vicia villosa*).—The plants were grown in large glass cylinders 60 cm. high and 10 cm. in diameter, having a volume of approximately 4 liters. In each of the cylinders were placed 250 cc. of the nutrient solution plus 1 per cent washed agar and galactose sugar. The cylinders were then fitted with cotton plugs and sterilized for one hour in an autoclave at a pressure of 15 pounds. The cultures were made in triplicate and the galactose was tested at 2 per cent and at 0.2 per cent concentration. After a growth period of 30 days the cultures showed the injurious action of the galactose, in each case the roots being markedly injured. The primary root tip coming in contact with the agar medium was killed and the lateral root produced met with the same injury, so that ultimately a multi-branched root system was produced after the manner of the pea roots shown in pl. 22 fig. 5. Whatever portions of the roots remained in contact with the agar medium were ultimately killed. It should be mentioned in this connection that the vetch grown in the presence of glucose, saccharose, lactose or maltose at concentrations of 2 per cent was greatly benefited. These sugars are absorbed and assimilated.

[1] Am. Jour. Bot. 2: 420–427. 1915.

Experiment with Canada field pea (Pisum sativum).—In the first experiment with the pea the large cylinders were again employed and to each were added 200 cc. of the nutrient solution plus 1 per cent agar and the sugar whose effect was to be tested. Cultures were made with raffinose, saccharose, lactose, glucose, and galactose ("Merck's Highest Purity"), the concentration of the sugar employed in each case being 2 per cent. The cylinders were fitted with cotton plugs as in the previous experiment and then sterilized for a period of one hour. In each cylinder were sown four peas which had

TABLE I

DATA ON CANADA FIELD PEA

(Duration 25 days. Taken February 13)

Culture	No. of plants	Height of plants cm.	Total green wt. grams	Dry wt. cotyledons grams	Dry wt. roots grams	Dry wt. tops grams	Total dry wt. grams	Av. dry wt. grams	Gain per plant grams
Glucose	3	44 40 38	6.250	.155	.170	.364	.689	.229	+.085
Lactose	4	40 40 33 33	6.700	.169	.105	.355	.629	.157	+.007
*Raffinose	4	32 33 24 33	6.500	.192	.130	.328	.650	.162	+.012
Saccharose	4	39 35 36 35	7.600	.160	.144	.430	.734	.183	+.036
Check	3	32 23 34	4.450	.150	.075	.190	.415	.138	—.012
Maltose	4	33 40 34 28	6.600	.222	.142	.386	.750	.187	+.034
Galactose	Plants small and roots injured. (See pl. 22, figs. 1a and 5.)								

* Reducing sugar formed in medium probably as a result of secretion of invertase and raffinase from roots. Acidity of entire medium at time of examination equivalent to 0.7 cc. N/10 KOH.

been sterilized by the method described. The plants were grown for a period of twenty-five days and then data taken on the various cultures. The various cultures are shown in pl. 22 fig. 1. The galactose plants are separately shown in pl. 22 fig. 5 and the detailed data are given in table 1.

An examination of the table reveals the fact that every sugar acted beneficially except galactose. If the plants had been examined a month later (as was the case with other cultures), much greater differences would have been secured between the check cultures and the sugar-containing cultures. Lactose is undoubtedly utilized by Canada field pea as well as by vetch and probably before assimilation is converted into glucose and galactose. Raffinose, which is also utilized, yields on hydrolysis first levulose and melibiose, and the latter is further transformed to galactose and dextrose. In the light of the foregoing, it would appear from the results secured with lactose and raffinose that levulose and glucose must exert some protective action against the injurious action of galactose.

Influence of concentration of galactose.—In all of the previous experiments the galactose sugar was employed at only two concentrations, namely, 0.2 per cent and 2 per cent. In the following experiment a series of cultures was made containing galactose at the following concentrations: 0.125 per cent, 0.25 per cent, 0.50 per cent, 1.0 per cent, 2.0 per cent, and control cultures lacking galactose. The plants were grown in large test-tubes 30 cm. × 4 cm., containing 50 cc. of the nutrient medium plus 1 per cent agar. The galactose sugar employed in this experiment was provided by Dr. C. S. Hudson[1], Chief of the Carbohydrate Laboratory, U. S. Bureau of Chemistry. The galactose sugar provided had been recrystallized and was stated by Dr. Hudson to be of a very high degree of purity and probably purer than any which could be secured upon the market. The tubes were plugged with cotton and sterilized in an autoclave at 15 pounds pressure for a period of 20 minutes. One pea was sown in each tube and the cul-

[1] The writer gratefully acknowledges his indebtedness to Dr. Hudson for the galactose furnished.

tures made in triplicate. The seeds germinated in four days and even by this time in the higher concentrations of galactose, browning of the cotyledons was becoming evident. This browning of the cotyledons intensified with time and at the end of 20 days the peas in the 1 per cent and 2 per cent galactose cultures showed marked discoloration, and death of roots soon occurred. The height of tops was also markedly affected in the presence of galactose of a concentration of 1 per cent or over. (See pl. 22 fig. 4.) The above experiments were repeated with wheat and corn and the results secured were similar.

Antagonistic action of glucose toward toxicity of galactose. —It was noted previously that raffinose and lactose are utilized by Canada field pea, and this has been verified by other experiments. The use of lactose by vetch has also been decidedly shown by experiments not yet reported. Since both lactose and raffinose are assimilated by pea and vetch, and since it is highly probable, as previously suggested, that these sugars are hydrolyzed before assimilation, it is possible that the glucose and levulose exercise a protective action against the galactose.

An experiment was made to test the hypothesis with respect to glucose. Test-tube cultures were prepared as in the previous experiment, but in this case were made in quadruplicate. One series contained 1 per cent glucose plus 1 per cent galactose and the second series contained 1 per cent galactose alone. The plants were grown for 25 days in the greenhouse and the general results are clearly evident in pl. 22 figs. 2 and 3. In the case of the 1 per cent galactose culture the primary roots were killed, but with the 1 per cent glucose added, the primary root tip was killed and the epidermis and part of the cortex, but the inner part of the root was not apparently injured, for secondary roots developed which seemed to be more resistant to the toxic action of the galactose, for these root tips suffered no injury and not even a browning of the root was secured as was the case with the primary root (pl. 22 fig. 3). The experiment was repeated a second time and the results secured are concordant with the first.

Discussion.—So far as the writer has been able to discover, no previous mention has been made of the toxic nature of galactose for plants. Molliard[1], however, intimates that galactose is toxic for radish, for unlike other sugars, the galactose permitted no development beyond a 5 per cent concentration and with 2 per cent galactose the plants are very small. He concludes that galactose is not utilized by radish.

That galactose is injurious to the green plants employed is definitely shown. It does not appear to be toxic to fungi since *Aspergillus niger,* several species of *Penicillium,* a species of *Fusarium,* and a species of *Mucor* were all found growing in cultures which became contaminated. It is definitely known also that certain yeasts are able to ferment galactose. The character of the injury effected by the galactose in the above experiment and the method of action have not yet been determined. Incidental observations indicate that the galactose on penetrating kills the cells in its path. In the case of peas grown on 1 per cent galactose the peripheral layers of the cotyledon showed the original starch reserve undigested. In the presence of glucose it was only the epidermis and part of the cortex which suffered injury. It would appear that the outer layers of cells were injured before sufficient glucose had accumulated to render them resistant to the toxic action of galactose, or perhaps the penetrability of the inner cells for galactose was altered by the presence of glucose. In what manner the glucose antidotes the toxicity of galactose cannot yet be stated. It may be possible that it is the oxidation products of galactose that are the injurious agents and that the glucose prevents the formation, or modifies the character, of the oxidation products and that the toxicity is thereby overcome. Investigation into other phases is in progress.

[1] Molliard, Marin. Action morphogénique de quelques substances organiques sur les végétaux supérieurs. Rev. Gén. de Bot. 19: p. 331. 1907.

Explanation of Plate

PLATE 22

Fig. 1. *a*, galactose 2 per cent; *b*, lactose 2 per cent; *c*, check—no sugar; *d*, raffinose 2 per cent; *e*, saccharose 2 per cent; *f*, glucose 2 per cent. (Cotton plugs were removed at the time of photographing.)

Fig. 2. The two outside tubes contain 1 per cent galactose while the two middle ones contain 1 per cent galactose plus 1 per cent glucose.

Fig. 3. The small plant was grown on 1 per cent galactose; the larger one on 1 per cent galactose plus 1 per cent glucose.

Fig. 4. *a* and *b*, 2 per cent galactose; *c*, 1 per cent galactose; *d*, 0.500 per cent galactose; *e*, 0.250 per cent galactose; *f*, 0.125 per cent galactose; *g*, check—no galactose.

Fig. 5. Peas (shown in fig. 1*a*), showing character of root growth when tips alone come in contact with 2 per cent galactose-containing medium.

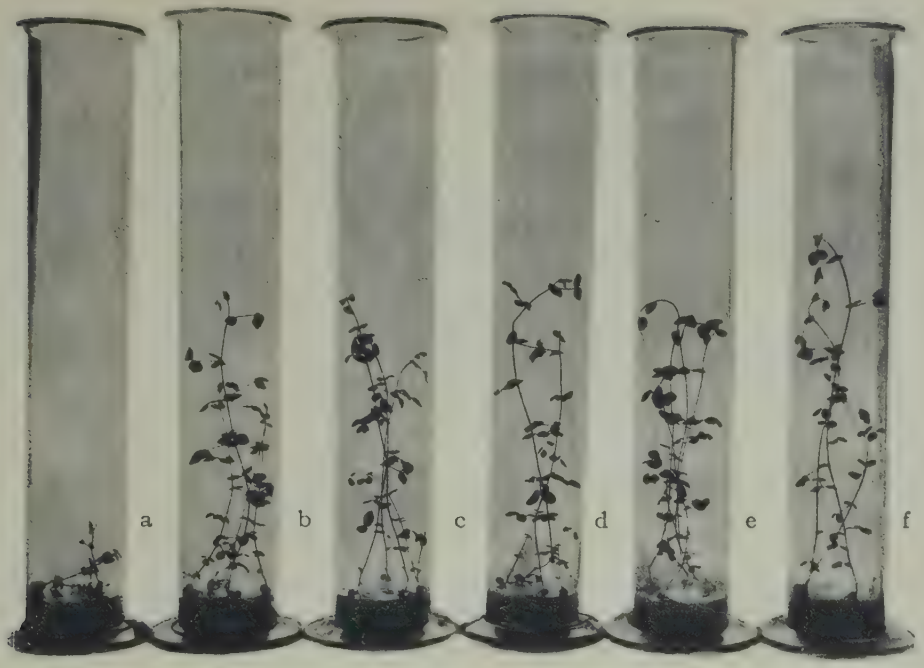

Fig. 1

Fig. 2

Fig. 3

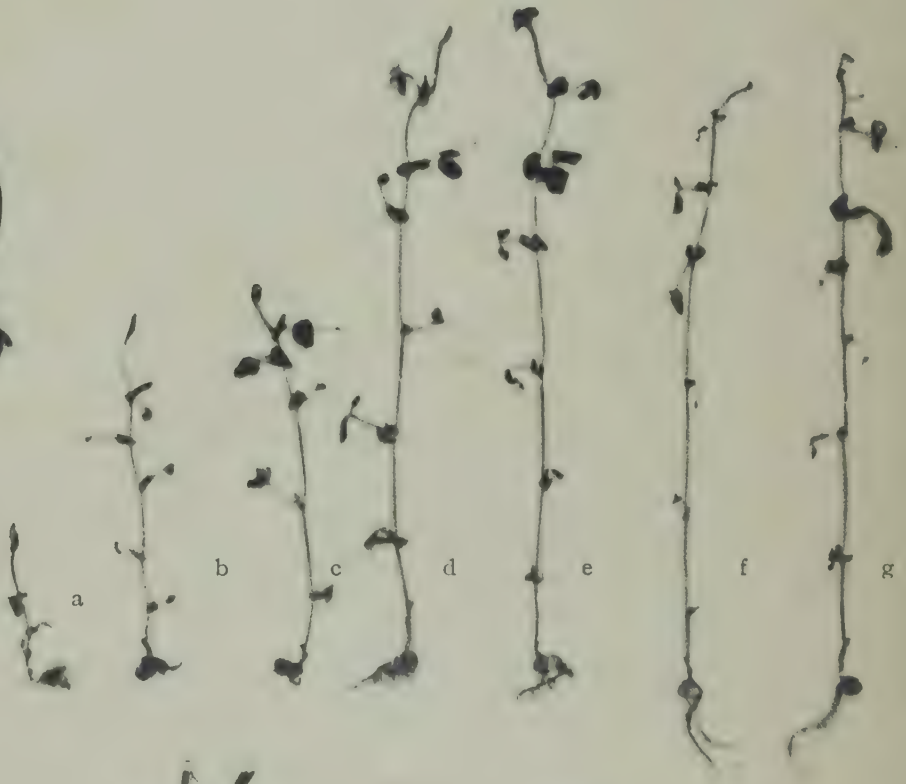

Fig. 4

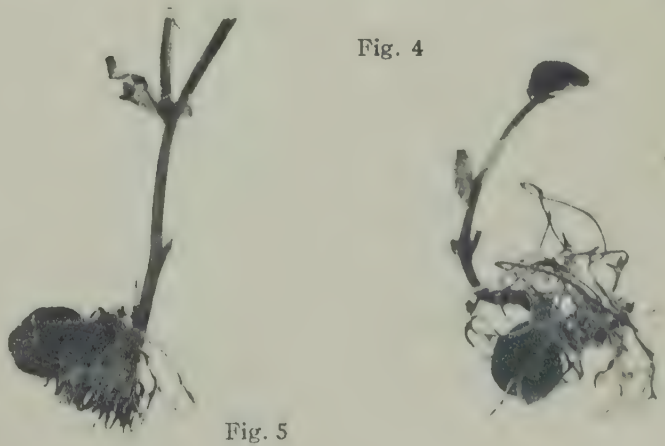

Fig. 5

COMPARATIVE STUDIES IN THE POLYPORACEAE

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The subclass *Basidiomycetes* of the class *Fungi* contains a natural group of plants sharply separated from related groups in that the hymenium (basidia, paraphyses, etc.) forms the lining of hollow tubes on the ventral surface of the fruit body. This group of plants constitutes the tribe *Polyporeae.* It is divided into two families, the *Boletaceae* and the *Polyporaceae.* The *Boletaceae* are separated from the *Polyporaceae* in that they are fleshy and soon decay and the tubes are easily separated from the pileus, while the *Polyporaceae* vary in texture from coriaceous to hard and woody, and the tubes are inseparable from the pileus. These characters are susceptible of some variation, as there are a very few fleshy species in the latter family, and in two or three cases the hymenium is waxy and the tubes separable. In this article we are concerned only with the *Polyporaceae.*

Historical

Accurate knowledge of the classification of the *Polyporeae* dates back only to the last few years of the eighteenth or the beginning of the nineteenth century. The first attempt worthy of consideration was that of Persoon in 1801, although we still have occasion to refer to articles by earlier writers, especially Bulliard (Herbier de la France, 1780–1793), Schaeffer (Fung. Bav. 1780), and Sowerby (Eng. Fung. 1797–1809). These three, while contributing considerable in the way of illustrations of the species known at that time, knew very little about the correct classification of the species they illustrated. The binomial method of naming species had come into general use following its introduction by Linnaeus (Species Plantarum) in 1753, and many new species were described in the succeeding years, but the descriptions were inadequate and

the type specimens not preserved, so that it is impossible to tell to what plants the descriptions refer.

By the beginning of the nineteenth century those interested in this line of study had begun to feel the need of permanent herbaria containing specimens of all the species described. The appreciation of this need augmented the demand for a more systematic and a more natural arrangement of the genera and species of fungi.

It thus came about that while Linnaeus in 1753 had listed but one genus, *Boletus,* and 12 species of pore fungi (*Boletaceae* and *Polyporaceae*), the number of genera had increased to 3 and the number of species to 93 when Persoon published his 'Synopsis Fungorum,' in 1801. This was followed by the work of Albertini and Schweinitz (Conspectus Fungorum) in 1805, which was modeled after the work of Persoon and contributes nothing to the systematic arrangement of the *Polyporeae.* It must not be supposed, however, that there was any extraordinary change from the incomplete descriptions of the earlier writers to a more or less perfect standard of description that should include all the facts necessary for the identification of the species. The descriptions in Persoon's 'Synopsis' were still far from what could be desired, and it is only where these are supplemented by herbarium specimens or by accurate illustrations or by both that the species can be identified beyond all doubt. But the fact remains that the beginning of the nineteenth century witnessed a growing inclination on the part of mycological systematists toward a form of record for the species that would be more concrete in its conception and thus give an added impetus to the study of the fungi.

Among the vast array of mycologists produced in the nineteenth century by far the most prominent was Elias Fries. His first work of importance was the 'Systema Mycologicum,' published in 1821–1832, in which the known fungi were marshalled in order. To the genera of the *Polyporeae* listed by Persoon he added the genus *Polyporus* (first proposed by Micheli in the eighteenth century) and thus made the first attempt to separate the *Boletaceae* from the *Polyporaceae.*

The genera treated by him contained 164 species in all, of which probably two-thirds were in the single genus *Polyporus.* This genus was divided into 3 sections, *Favolus, Microporus,* and *Polystictus,* the first named being later raised to generic rank. The section *Microporus* contained by far the largest number of species. It was divided into 5 subgenera: *Mesopus, Pleuropus, Merisma, Apus,* and *Resupinatus.* This arrangement was continued in his 'Epicrisis Systema Mycologicum,' published in 1836–38. In the meanwhile the genera *Trametes, Cyclomyces, Hexagona, Favolus, Laschia,* and *Porothelium* had been carved from the old genus *Polyporus,* and the number of species described had increased to 361 (entirely exclusive of the genus *Boletus*). Of these, 280 were included in the genus *Polyporus.* The same disposition of the pore fungi was followed by Fries in his last publication, 'Hymenomycetes Europaei,' in 1874, and, indeed, that system has either been followed in its entirety since or has served as a foundation for all other systems of classification that have been proposed from time to time by others.

Correlated with the increase in the number of described species there is manifest a tendency on the part of some later writers toward a change in the conception of what should constitute a genus. There has been a tendency away from the old idea of large genera containing a heterogeneous collection of species, and toward the breaking up of genera into smaller units consisting of closely related individuals. This tendency finds its best expression in the work of Karsten, Quelet, and Murrill, each of whom has published papers dealing with the classification of the *Polyporaceae.*

IMPORTANT MICROSCOPIC CHARACTERS USED BY EARLIER WORKERS

Having glanced at the beginnings of the various classifications that have been proposed, we may now turn our attention to an analysis of the characters used in separating genera and species. For the most part the generic characters were macroscopic ones, such as presence or absence of a stipe, consistency of the sporophore, nature of the hymenium, etc.,—characters that arrested the attention of the collector without

recourse to the microscope, for the microscope was unknown when the foundations of this study were laid. In the separation of species other macroscopic characters of minor importance were used. Color, pubescence, habitat, form, size, etc., were characters that were largely drawn upon in fixing the limits of species.

It was unfortunate, however, that though the characters named are the most conspicuous ones, yet they are more subject to modification and variation than are certain internal characters that require the use of the microscope for their detection. Perhaps the desideratum in systematic botany would be a classification in which genera are well defined and sharply separated from each other by gross morphological characters, and in which the microscope would be necessary only in determining specific characters. Perhaps this demand is more nearly filled in the family *Agaricaceae* than in any other group of the fungi. There the genera are divided into sections on the color of the spores, and the genera in these sections are more or less well differentiated on gross morphological characters.

In those groups of the fungi that have been most carefully studied, e. g., the *Myxomycetes,* considerable attention has been paid to the minute anatomical structure of the plant. Spore markings that are scarcely visible, except with an oil-immersion lens, have been used as points of separation in closely related forms, and in certain of the *Discomycetes* the spore markings and the nature of the paraphyses have been largely drawn upon to furnish specific characters. Durand[1] has gone somewhat farther, and in his studies in the fleshy *Pezizineae* has taken into account the structure of the apothecium in fixing the limits of the families. Burt[2] has recently set new limits to some of the genera of the *Thelephoraceae,* in keeping with their inner anatomical structure. In the *Polyporaceae,* Miss Ames[3] has recently attempted to outline a scheme of classification of the genera based largely on the structure of the sporophores, but only a few forms

[1] Bul. Tor. Bot. Club **27**: 463–495. 1900.
[2] Ann. Mo. Bot. Gard. **1**: 195–196. 1914.
[3] Ann. Myc. **11**: 211–253. 1913.

were investigated and the results not as satisfactory as could be desired.

It is a significant fact, however, that no attempt has been made to classify the *Polyporaceae* on the basis of spore or other hymenial characters, although it is recognized that, outside of the algae, the organs concerned in reproduction are usually subject to less variation than are external morphological characters. That no such attempt has been made is due to two causes: first, the dislike on the part of students of the careful and painstaking observations that must often be made to determine those characters; and second, to the widespread belief that the pore fungi are spore-bearing only for a short interval of time during the year, and that they must be examined at the right moment or the spores will have disappeared. When it has been shown that the second objection is invalid and that hymenial characters are usually not hard to make out, the first objection will largely disappear.

In the course of the last year the writer has spent a considerable portion of his time in searching for these characters, not only in the *Polyporaceae* but in other related families as well. The methods employed are given on a following page, and suffice it to say here that probably 75 per cent of the collections examined contained spores, and a large percentage afforded other microscopic characters that played a considerable part in distinguishing one species from another. The characters that may be obtained by the use of the microscope are here enumerated and some indication given as to their possible value.

Discussion of Microscopic Characters Now Available for Use as Generic and Specific Characters

The characters that may be obtained by the methods outlined on a following page are as follows: spore characters, presence or absence of cystidia, setae and other sterile organs in the hymenium, basidial characters, hyphal characters, and the presence or absence of sterile structures in the subhymenial tissue.

Spore characters.—Spore characters are probably worthy of a great deal more consideration than they have yet received in the greater part of the mycological work that has been done up to the present time. As previously stated, in the *Agaricaceae* the primary divisions of the family are made on the basis of spore colors. This distinction was made as early as 1821 by Fries in his 'Systema Mycologicum.' The fact that this character was so early recognized was not because spores are more abundant or their colors more striking in the gill fungi, but because the period of spore production more closely coincides with the period of maximum development of the plants. Unfavorable conditions, i. e., drought, superabundance of moisture, cold, etc., result in the disorganization of the tissue in a fleshy fungus, and consequently the duration of the period of spore liberation is permanently shortened. In the coriaceous or woody forms these same conditions result only in a temporary suspension of the act of spore liberation and with the return of normal conditions the suspended function again becomes active. In this way the period during which spores are present in the hymenium of a pore fungus is greatly lengthened, and it is safe to assume that the number of mature spores present at a given time in the hymenium of one of the more durable pore fungi is less than the number of mature spores on an equal hymenial surface of a gill fungus. Contrary to the condition in the *Agaricaceae,* the introduction of spore colors as generic characteristics would mean an entire revision of all the genera, and it may well be doubted whether the advantage obtained from such a limitation of genera would compensate for the confusion that would be sure to arise. On this basis, however, the species could easily be grouped into sections under the genera, but even were that done the white-spored species so far outnumber those with colored spores that the adoption of the idea would delimit only a small group of species that perhaps could be better separated in other ways.

Very little exact evidence bearing on the variation in size in the spores of a given species is obtainable. The work of

Falck[1] showed that the mature spores of certain species of *Lenzites* were very constant in the length of their short axes, the variations being only a fraction of one micron, while the length of the long axis varied considerably, although in that case the variation rarely went beyond 3 μ in different spores from different fruit bodies. Cotton[2] investigated variations in the spores of *Stropharia semiglobata* and found that when the pileus was cut from the stem and a series of spore prints obtained from the former, the spores shed during the first hour measured 18×10 μ, while those shed during the twenty-third hour measured 15×9 μ, and those shed during the eighty-third hour measured only 12×7 μ. The diminution in size was ascribed to the artificial conditions, i. e., the pileus being severed from the stipe, under which the spores were produced. Experiments carried on with sporophores collected and placed in large test-tubes and supplied with water, showed that the spores shed the first day did not differ in size from those shed during the fifth or sixth day. The first experiment suggests the possibility that in plants growing in nature the size of the spores might be reduced if the fungus was growing on a substratum in which the required amount of food substances was not present. No comparative studies along this line have yet been reported and the question of the amount of variation in size of spores is still an open one. However, spore measurements have been very successfully used in separating species of fungi and no doubt the limit of their usefulness has not yet been reached in systematic mycology.

Inaccurate spore measurements may creep into the literature through a misdetermination of species quite as easily as species may be misdetermined because of inaccurate spore measurements. The former condition is especially liable to be pronounced in the literature of a fungous flora as little known as is that of this country, and where species are not determined on microscopic characters, but these same characters are entered in the literature when the species is re-

[1] Moeller's Hausschwamm-forschungen, Heft 3, pp. 79–96. 1909.
[2] Trans. Brit. Myc. Soc. 4: 298–300. 1914.

corded. This latter procedure is entirely commendable, but it has been so much abused that the spore characters carried in the literature are far from being reliable in a large number of cases. However, allowances must be made for some variation in measurement by different individuals as no two persons will report exactly the same measurements for one species.

The shape of the spores is probably subject to somewhat less variation with age than is the size. Spores begin to take their characteristic shape while they are yet comparatively immature and from seeing such a spore one can judge of its mature form more accurately than of its mature size. Often the spores of two or more species are so similar in shape that it is perhaps best not to try to distinguish between them, although the distinction may be perfectly apparent to one who has before him the spores of all the species in question. The terms used to describe spore forms are not as rigidly defined as we could wish, and it does not add to the clearness of distinction between two species to describe the spores of one as "elongate-ellipsoid" and of the other as "narrowly fusoid" and expect the users of the manual to distinguish the species on that basis. There are many cases, however, where the form of the spores may be used to good advantage.

Spore markings are so universally absent in the *Polyporaceae* that the subject requires very little comment here. There are probably not more than a dozen species that are characterized in this way and they are so widely separated that the character is given an added value. In some groups of the fungi, especially among the *Ascomycetes,* not only the presence or absence of markings on the spore wall but also the nature of these markings is taken into account.

Cystidia.—Cystidia may be defined as more or less conspicuous sterile organs found either in the hymenium or in the subhymenial tissue of various basidiomycetous fungi. They are usually unicellular and they may be smooth or they may have a more or less incrusted surface, the incrusting substance probably always being calcium oxalate. The name "setae" has been given to these bodies when they are colored

(usually brown) and sharp-pointed, and that distinction is maintained in this paper, although there may be some doubt as to the advantage that accrues from its use. The presence or absence of setae has been made a generic character in some groups of the *Basidiomycetes,* and even in the *Polyporaceae* the genus *Mucronoporus* was founded by Ellis and Everhart on the presence of the setae in the hymenium. The genus probably has not received the acceptance that it has deserved at the hands of mycologists. It is difficult to say at times whether a given structure should be designated as a cystidium or not, but the writer is of the opinion that the term should be used in its broadest sense, except that it should not be applied to those structures usually referred to as paraphyses. These latter can usually be distinguished by the frequency of their occurrence as they usually alternate with the basidia, while cystidia or setae are scattered irregularly through the hymenium. In by far the largest number of cases the cystidia are very conspicuous on account of their size, coloration, incrustation, or other characters. In a few cases the presence or absence of setae is a variable character, in some specimens being abundant and in others very scarce. In such cases the writer has found it advisable to make longitudinal sections of the tubes, as the setae are sometimes more abundant in one part of the tubes than in another. A cross-section of the tubes of *Fomes igniarius* will sometimes fail to show a single seta, but in only one specimen has the writer failed to locate them in longitudinal sections from the hymenium of the same plant. They are also almost entirely lacking in some specimens of *Polyporus dryophilus.*

Basidia.—It is very seldom that the basidia offer characters that can be used in separating species. They are almost universally 4-spored in the *Polyporaceae* and in those few species where 2- and 3-spored basidia do occur there are always a goodly number of 4-spored ones present also. In a very few cases the basidia are conspicuous on account of their large size. This is true of *Trametes Peckii* where they are 8–10 μ broad, while usually they vary from 3 to 6 μ broad.

Hyphal characters.—The characters of the hyphae that make up the subhymenial tissue and the tissue of the trama of the pileus have never been used in the classification of the *Polyporaceae.* While the size of the hyphae may depend to a considerable degree on the food supply of the plant, yet in examining a large number of species the writer has found that some are characterized by hyphae two to three times as large as in most species. These cases have been thoroughly investigated as far as herbarium material would permit and as all specimens have showed the character about equally well, it has been taken as a means of identifying the species in which it has occurred. The writer knows of no factor or combination of factors that would be operative on a large number of individuals from widely separated localities and in the case of but a limited number of species. If it be dependent on nutrition, then the species possessing this character are so constantly associated with that kind of nutrition that the character is as constant a one as can be obtained. The same is true of the unbranched hyphae of the context of *Polyporus albellus.*

Incrustation of the hyphae has never been observed in the pileate *Polyporaceae,* though it is a well-marked character in the species of certain groups of resupinate fungi.

METHODS EMPLOYED

A few words may not be amiss here concerning the methods employed by the writer in obtaining these microscopic characters. In general the method is that already described in a previous number of this journal.[1]

Obtaining spore prints.—In the case of fresh specimens just brought into the laboratory from the field, spore prints are very easily obtained by placing the specimens on a glass slide in such a manner that the tubes are in a perpendicular position so that the spores do not lodge on the sides of the tubes when they are liberated from the basidia. The slide with the fungus in position should be either wrapped in waxed paper or left over night or for several hours in the collecting

[1] Burt, E. A. *loc. cit.*

basket or other receptacle in which a fairly high humidity will be maintained, so that the liberation of the spores will not be prematurely stopped by the drying-out of the tissues of the fungus. If the specimens are dry when brought into the laboratory they may be moistened thoroughly with water and then treated as described above. One unaccustomed to this procedure will be surprised to find how large a percentage of the collections so treated will produce a good spore print. Specimens collected on the warm days that frequently come in January and February have often been treated in the above manner with gratifying and surprising results. When desiccation takes place by exposure to the air the vitality of many species is not destroyed. Buller[1] was able to restore normal vitality to such plants by placing wet cotton-wool on their upper surfaces. He was even able to revive the fruit bodies of *Daedalea unicolor* after they had been exposed to ordinary air at room temperatures for eight years and three months, and of *Schizophyllum commune* after an exposure of six years and three months. In most species, e. g., *Polyporus versicolor, P. hirsutus,* and *Lenzites betulina* the vitality was retained for a period of but two to three years.

Sectional preparations.—In case one is working with material that has been in the herbarium for several years the above method will not answer. Neither does it furnish any evidence as to the other microscopic characters of the plants. One must then resort to sectional preparations. These are cut free-hand with a very sharp sectioning razor. Free-hand sections are quickly made and the results from them are usually better than from microtome sections. It is impossible for the spores to retain their position on the basidia when subjected to the different processes involved in preparing material for microtome sectioning. The first requisite in successful free-hand sectioning is material in good condition; the second is a very sharp razor (preferably flooded with alcohol); the third is some little skill and experience. The hymenium of the specimen is first moistened with alcohol, then with water,

[1] Researches on fungi, pp. 105–111. 1909; and in Trans. Brit. Myc. Soc. 4: 106–112. 1913.

and a piece about 2 mm. square on the hymenial surface is cut out with a scalpel. If material is abundant the process may be reversed and a larger piece than needed may be cut out with the scalpel, trimmed to the requisite size, immersed in 95 per cent alcohol for a few seconds and then transferred to water. In the writer's experience the latter method is the more preferable and has probably been the one most used. The material does not soften while in alcohol, but that reagent is used only to facilitate the absorption of water by the tissue. Any rigidity that may be imparted to the tissue by the alcohol is probably overcome when the material is transferred to water. In some cases when this transfer is made the tissue either becomes very soft or very friable so that no razor, however keen, will cut a clean section through it. It is here that the latter method obtains preference over the former, for after some experience one can judge of the probable effect the water will have and by shortening the period that the material remains in the water the tissue is in better condition for sectioning.

The most instructive preparations are often those containing both longitudinal and cross-sections of the tubes. Such sections are easily obtained in one mount by cutting out the piece of material somewhat longer in one direction than stated above—say about 2×4 mm. on the surface. Several longitudinal sections may be cut from this and the position of the remaining bit of tissue so changed that cross-sections may be obtained.

For sectioning, the tissue is placed in the proper position in a piece of pith and as the sections are cut they may either be transferred directly to the slide by means of a camel's-hair brush dipped in alcohol, or they may be allowed to accumulate in the alcohol on the razor and then flooded off into a watch-glass containing alcohol. By the last method one can pick out with more accuracy the thinner sections by observing them under the lens of a low-power dissecting microscope. The writer has found it to be sufficient in most cases to transfer the sections directly to the slide, disregarding the thicker sections that are cut, or brushing them off the edge of the

razor with an outward stroke of the finger. The sections are placed in a drop of 7 per cent KOH solution on the slide. This immediately expands the hyphae of the tissue to their normal size. The KOH solution is then drained off and a drop of stain added.

Staining and mounting.—I have tested a considerable number of the more common stains and so far I have failed to find one that gives universally good results if the sections are to be made into permanent mounts. For temporary mounts there is nothing superior to a 1 per cent water solution of eosin, but when sections so stained are mounted in glycerin the color soon completely disappears. The same strength solution of alcohol eosin (in 95 per cent alcohol) often gave a good permanent stain but quite as often it, too, faded out in the course of several weeks, and when used it gives a precipitate that must be washed off with water before the cover glass is applied. Why this stain should remain permanent in some cases and not in others is a question that has not been answered. It may be due to the KOH that remains on the slide and in the sections, but flooding the sections with water after draining off the KOH solution did not seem to have any beneficial effect. Different strengths of alcohol were used in preparing the stain, but with alcohols weaker than 95 per cent the stain disappeared even more quickly and the precipitation obtained was so great that such stains were of no value. From the facts observed it seems more reasonable to suppose that the difference may be in the tissue of the fungus rather than in the stain or the glycerin. A solution containing equal parts of a 1 per cent water solution and a 1 per cent alcoholic solution of eosin gave no better results.

Magdala red, Congo red, neutral red, acid fuchsin, methylen blue, and saffranin T were used, and of these, only the last one gave a permanent stain and it has been used in a large part of the work. It is a rapid stain, though probably not quite so rapid as alcoholic eosin, and it is well to leave the stain on the sections for about one minute. A 1 per cent alcoholic solution was used, the stain being dissolved in 95 per cent alcohol. When a drop of this stain is added and drained

off, the sections must not be allowed to become dry or an orange precipitate is obtained that necessitates the addition of alcohol to dissolve it. This also dissolves the stain from the tissues and the sections must be restained. This precipitate is not formed if a little water is added to the stain after it is made up. This stain imparts a uniform dull red color to the tissue but the color brightens when glycerin is drawn under the cover glass. Since it is not a differential stain its use is not advised where only temporary mounts are desired. It gives best results with very thin sections or with sections in which the hyphae are loosely arranged.

After the cover glass is applied the sections are ready to be examined under the microscope, but if the saffranin T stain is used, it is better to place a drop of glycerin at one side of the cover glass, at the same time drawing off some of the surplus water from the opposite side by means of filter paper. Several slides of each species are retained and mounted in 66 per cent glycerin. After a week or more all traces of the glycerin are removed from near the outer edge of the cover glass by means of a soft cloth dipped in 95 per cent alcohol. The slides are then ringed with some suitable cement—gold-size being most often used—labeled, and filed away in order. It will usually facilitate subsequent examination of the slides if the spore characters for each species are written on a slip of gummed paper and glued to one end of the slide.

It is sometimes quite impossible to find spores in the sections treated in the manner outlined above, since they are often easily removed from the sterigmata and washed away before the cover glass is applied. To overcome this difficulty the writer sometimes finds it advisable to distribute between two slides the sections obtained, one slide to be treated as outlined above, the other to be mounted for temporary observation only. This last one should be stained with a water solution of 1 per cent eosin, a drop of the solution being added to the drop of KOH containing the sections. Sometimes the staining is unnecessary, especially if one is dealing with species which have colored hyphae and colored spores. A

preparation made in this manner will often show spores when other methods of demonstrating them have failed.

Even with the most careful manipulation one will sometimes fail to find the spores, and, indeed, some species seem to be almost always sterile. In the case of *Fomes fomentarius* I cut sections of all the specimens available, and only when as a last resort, I sectioned a small and very unpromising specimen did I find the spores. I have been able to locate them in but one of the few specimens of *Polyporus graveolens* that were available for examination.

As stated above, the literature dealing with American *Polyporaceae* contains many inaccurate observations concerning spores. This is due mostly to a lack of care in making sure that a given body in the hymenium is really the spore of the fungus in question. The writer is of the opinion that spores should not be recorded for a collection unless they are obtained from a spore print or are seen attached to basidia. The spores found on basidia are usually somewhat immature, at least as regards size, but from their shape one can judge whether the spores found free-floating in the mount have any relation to the species under consideration. Where such free spores alone are present there is always the possibility that they belong to some other fungus and they should not be taken into consideration unless present in large numbers. One must also guard against the fact that the cut ends of hyphae may be in such a position as to appear globose in form and such may be mistaken for spores.

Examining the context hyphae.—In obtaining the characters of the hyphae of the context a bit of tissue is picked out with the forceps and mounted on a slide in a drop of KOH solution. In the case of some of the species of the genus *Fomes* where the context is hard and woody, it is usually better to boil a bit of the context in a KOH solution for a few minutes. In this way the tissue is softened and when teased apart on the slide with needles, a cover glass added, and pressure applied, the hyphae will generally separate out so that their characters may be obtained. In all cases the

hyphal measurements given are for the hyphae in the context of the plant and not for those in the subhymenial tissue.

Statement of Problem

The writer presents in this paper the results obtained by carefully investigating some of the more common species of pore fungi, using the methods outlined on the preceding pages. There are certain groups of species in the *Polyporaceae* that are very much in need of just such treatment, and it is to these groups that the writer has turned his attention. The groups consist of closely related species that have been separated heretofore largely on external characters and in a great many cases the results have only led to confusion. The problem, as the writer saw it, was one involving a contribution toward a more exact characterization of these species and their separation, wherever possible or feasible, on some constant internal microscopic character. Some species are well enough marked by external characters so that such distinctions should be used only as supplementary characters, while in other cases the characters obtained by this study should displace those hitherto used.

The results obtained were not as gratifying as was expected when the work was undertaken. Only a small beginning has been made, for it is a laborious task involving the cutting and examination of many sections for each species in order to be sure that the characters shown by the first sections are constant for all collections of the same species. The work should be carried on although several years would be required for its completion. Permanent mounts of the sections have been made for each species and these are available for future reference. Criticisms and suggestions, both of methods employed and results obtained, are invited and will be given careful consideration.

Acknowledgments

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his valuable herbarium; to Dr. B. M. Duggar for his interest in the work and for aid in various ways; to the Missouri Botanical Garden for the use of the herbarium material and library facilities; to Mr. C. G. Lloyd of Cincinnati, Ohio, for aid in determining some of the species.

Polyporus abietinus Dicks. ex Fries and P. pargamenus Fries

P. abietinus was first described by Dickson,[1] in 1793, and appears to be almost cosmopolitan in its distribution. In the United States it is found wherever coniferous forests abound, from Canada to the Gulf of Mexico, and from the Atlantic to the Pacific Ocean. It is never found on the wood of deciduous trees, and as will be pointed out later, this fact affords almost the only constant character by means of which it can be separated from its near relative, *P. pargamenus.*

P. pargamenus was described by Fries,[2] in 1838, from plants collected on pine wood in Arctic America by the Franklin Expedition. The plant has not been reported from the western coast of the United States, but has been found in practically every state east of the Mississippi River, ranging west as far as Wisconsin, Kansas, Arkansas, and Colorado. It is also found in Europe. Most of the collections in this country under the name *P. laceratus* Berk., *P. xalapensis* Berk., or *P. ilicincola* Berk. and Curt., belong to this species. An examination of *P. pseudopargamenus,* as distributed by de-Thuemen,[3] shows it to be identical with *P. pargamenus.* The writer has not seen authentic specimens of the other species named above, but they are given as synonyms by Murrill.

By some writers the two species have been confused, due to the fact that the type specimens of *P. pargamenus* were reported as growing on the wood of coniferous trees, while in the United States the plant that has gone under the name *P. pargamenus* is confined entirely to the wood of deciduous trees. This has led some authors to regard the original *P.*

[1] Pl. Crypt. Brit. 3: p. 21. 1793.
[2] Epicr. Syst. Myc. p. 480. 1838.
[3] Myc. Univ. 1102.

pargamenus as probably a synonym for *P. abietinus*. In that event, the species on the wood of deciduous trees would have to be given another name. This point can be settled only by a study of the type specimens of *P. pargamenus,* if they are still preserved. Nearly all the exsiccati material has been distributed under the name *P. pargamenus,* and the plant is so common and the name so well established that it is the writer's opinion it should not be changed without recourse to the types.

The two species under discussion are very closely related and they are connected by intermediate forms to such an extent that it is difficult to refer some collections to their proper species. However, the usual form of the fructification is distinct enough. *P. abietinus* is usually much smaller, is frequently effused-reflexed with a narrow and often laterally continuous pileus, rarely more than 2 cm. in length, and the tubes sometimes break up into lamellae-like plates—a condition I have never found in *P. pargamenus*. That species often grows much larger than *P. abietinus,* sometimes attaining a length of 6–7 cm., and is often fan-shaped or cuneate in outline and attached by a narrow, attenuate, sometimes stem-like base, so that the form and size of the fruiting body will usually separate it from *P. abietinus*. The color, zonation, and pubescence of the pileus is similar in both species, though the pubescence is often inclined to be strigose in the latter plant and more velvety in the former. Both species often have a violaceous or lavender tint to the hymenium or on the margin of the pileus.

The microscopic appearance of the hymenium of the two species does not furnish additional characters for their separation. The spores are similar in size and shape, being cylindric or sometimes allantoid, hyaline, smooth, and measuring 5–7 $\times$ 1.5–2.5 μ (not globose, 4.5–5.5 μ as stated by Murrill). Murrill states that no cystidia are present in the hymenium of *P. abietinus* and to the writer's knowledge their presence has never been recorded. I have examined several collections of both *P. abietinus* and *P. pargamenus* and I find that the plants vary as regards this character. I am of the opinion that cystidia are probably always present, but at times are so rare

or so inconspicuous that close observation is necessary to detect them and I have often examined whole sections without being able to locate them. A similar section taken elsewhere in the hymenium may show an abundance of them. The accompanying illustrations (figs. 1 and 2) show the different forms they may assume, but perhaps the most common form is as shown in *a* of fig. 1. They are often scarcely larger in size than the basidia, but are different in shape, usually with the appearance of slender pegs tapering to a rather blunt point. Rarely they are somewhat fusiform in shape and reach a length of 20 μ and a thickness of 6 μ. These sizes are unusual, however. They are colorless or almost so, sometimes scarcely extending beyond the basidia, but sometimes projecting enough that one can easily pick them out with the low power of the microscope. They are usually unincrusted, but sometimes their tips are somewhat capitate with small crystals (see fig. 2). They are then much more conspicuous, and in some collections this appears to be the predominating condition.

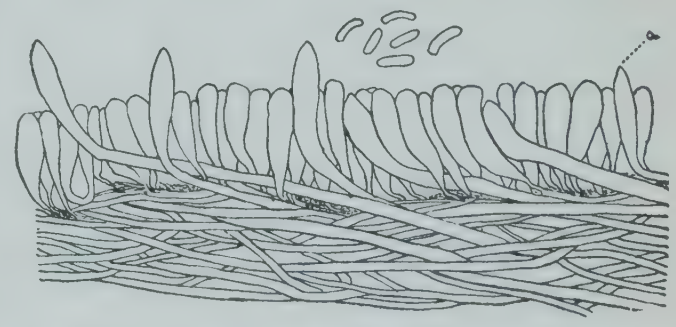


Fig. 1. Section of the hymenium of *P. abietinus* showing cystidia and spores.

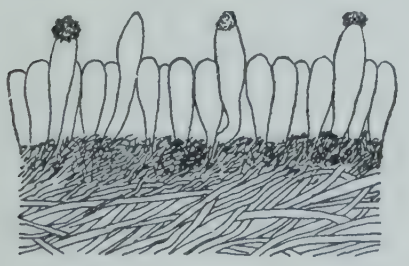


Fig. 2. Section of the hymenium of *P. abietinus* showing cystidia incrusted at the apex.

Before the writer had seen this more conspicuous type it was thought these sterile, inconspicuous structures might be basidia that had discharged their spores and had thus been rendered hyaline, as it is frequently found in other species that the mature spore-bearing basidia project somewhat beyond those that have not reached maturity. The shape of these bodies and the fact that they often assume a capitate apex, as do cystidia of many other species, make this view untenable. If more proof were needed it might be pointed out that these bodies are present in young specimens and in

the growing margins of mature specimens where it is evident that no mature basidia have yet been formed.

Neither can these structures be regarded as paraphyses that have become elongated and, therefore, more conspicuous. While there may be no ground for the belief that paraphyses can not assume such a form, yet there is no evidence to indicate that conspicuous sterile structures ever have arisen in such a manner. Moreover, the distribution of these structures under consideration makes impossible any such idea, as they are scattered promiscuously and do not alternate with the basidia.

These two species then are to be distinguished only by their habitat, and the size and shape of the pileus. In my own collecting experience the former character alone is enough to separate them, but when once the two plants are learned, the matter of form and size will usually be sufficient for the identification of the specimens, even if the habitat be unknown.

As stated above, the hymenium of *P. abietinus* may at times be lamellate. This statement is made only after a careful study of the facts in the case. They are as follows: There is a plant with apparently the same distribution as *P. abietinus,* in which the hymenium is entirely lamellate. No exactly intermediate conditions have ever been seen by the writer, though he has collected both forms in Colorado. In all other characters the two plants are precisely similar. The host is always the wood of coniferous trees; the pubescence and coloration of the pileus is the same; the spores and cystidia are similar; and the hymenium often has the violaceous tint characteristic of *P. abietinus. Irpex fuscoviolaceus* is in all probability only another form of the same plant, although I have never seen specimens of that species with the well-marked lamellate hymenium of this form. The illustration (pl. 23 fig. 1) is from specimens communicated by Prof. C. R. Orton, of State College, Pennsylvania. He writes that the rot produced by this fungus is almost identical with the one produced by *P. abietinus.* Patouillard[1] represents the cystidia of *Irpex*

[1] Hym. Eur. *pl. 3. f. 23.* 1887.

fuscoviolaceus as incrusted at the apex in the same manner as shown in the accompanying illustration of *P. abietinus.* I have also found this condition to be predominant in the lamellate form of our species.

The following comparative synopsis of the two species discussed in this section is appended here:

1. Polyporus abietinus Dicks. ex. Fries.

Plate 23, figs. 1, 2.

Pileus coriaceous, *sessile or effused-reflexed,* 0.5–5 × 0.5–5 × 0.1–0.2 cm., white, cinereous, or blackish with age, villous, zonate; context not more than 1 mm. thick; tubes not more than 3 mm. long, the mouths white, bay, or violaceous, averaging 2–3 to a mm. in poroid forms, *but sometimes entirely lamellate;* spores cylindric or allantoid, hyaline, 5–7 × 1.5–2.5 μ; cystidia present or inconspicuous, hyaline, rarely incrusted at the apex, 3–6 μ in diameter, projecting 5–15 μ; hyphae of context hyaline, 3–4 μ in diameter.

On wood of *coniferous* trees, especially of *Pinus.*

Illustrations: Dicks. Pl. Crypt. Brit. **3**: *pl. 9. f. 9.*—Fl. Dan. *pl. 1298, 2079. f. 2.*—Gill. Champ. Fr. *pl. 463.*—Swant. Brit. Fung. *pl. 33. f. 2–3.*

Specimens examined: Barth. Fung. Col. 3108.—Cooke, Brit. Fung. 512, 605.—Thuem. Myc. Univ. 6, 706.—Ell. N. Am. Fung. 8.—Ell. & Ev. Fung. Col. 303.—Krieg. Fung. Sax. 1205.—Rab.-Wint. Fung. Eur. 3235 (as *Irpex fuscoviolaceus*).—Rav. Fung. Am. 422; Fung. Car. I, 12.—Shear, N. Y. Fung. 307.—Mo. Bot. Gard. Herb. 4726, 4727, 4728 (Newfoundland), 3854, 4213 (New York), 4214 (Labrador), 4220 (Alabama), 4074 (Colorado).—Burt Herb. (collections from Vermont and Washington).—Overholts Herb. 2001 (Colorado), 2465 (Pennsylvania), 2472 (Maine).

2. Polyporus pargamenus Fries. Plate 23, fig. 9.

Pileus coriaceous, *sessile, often narrowed at the base,* 1–7 × 1–7 × 0.1–0.4 cm., whitish, cinereous, or brownish with age, villous or *velvety-pubescent,* zonate; context less than 1 mm. thick; tubes not more than 3 mm. long, the mouths white, bay, or violaceous, averaging 2–3 to a mm. in poroid

forms *but usually soon irpiciform;* spores cylindric or allantoid, hyaline, 5–6 × 1.5–2.5 μ; cystidia present or inconspicuous, hyaline, rarely incrusted at the apex, 4–5 μ in diameter, projecting 5–15 μ; hyphae of context hyaline, 4–5 μ in diameter.

On wood of *deciduous* trees.

Illustrations: Freeman, Pl. Dis. *f. 36.*—Hard, Mushrooms, *f. 345.*

Specimens examined[1]: Barth. Fung. Col. 2825, 2924 (as *Coriolus prolificans*).—Ell. N. Am. Fung. 312.—Ell. & Ev. Fung. Col. 302.—Rav. Fung. Am. 423, 108 (as *Irpex fusco-violaceus*).—Rav. Fung. Car. I, 13.—Rab.-Wint. Fung. Eur. 3331.—Shear, N. Y. Fung. 38.—Thuem. Myc. Univ. 1102 (as *P. pseudopargamenus*).—Mo. Bot. Gard. Herb. 4086 (Missouri), 4431 (Arkansas), 3855 (New York), 4443 (Indiana), 4439 (Kentucky), 4433 (Illinois), 4436 (Alabama), 4559 (Georgia), 4557 (Florida), 42875 (New Hampshire).—Burt Herb. (collections from Pennsylvania, Vermont, Kansas, and Massachusetts).—Overholts Herb. 476, 269, and others (Ohio), 1756 (Colorado).

Polyporus adustus Willd. ex Fries, P. fumosus Pers. ex Fries, P. fragrans Peck, and Related Species

Perhaps no species have been more confused in American mycology than these three, together with a few other closely related forms both of Europe and America. They all agree in the one character of having a hymenium that usually becomes more or less smoke-colored at maturity. In *P. adustus* and its closest relatives, *P. crispus* Fries and *P. Burtii* Peck, the hymenium is usually black or grayish black from the first, while in *P. fumosus* and *P. fragrans* it frequently becomes

[1] Ell. & Ev. Fung. Col. 804, distributed as *P. pargamenus,* is *P. hirsutus* (certainly not *P. pubescens* as stated by Lloyd, Letter No. 52, p. 20). Ell. & Ev. N. Am. Fung. 1934, distributed as *P. pargamenus,* is not this species. The appearance of the plant suggests a form of *Irpex tulipifera.* I have made a microscopic study of the hymenium of the specimen and I find it has the larger incrusted cystidia of that species and not the inconspicuous cystidia of *P. pargamenus.* Mycological literature contains several names for plants closely related to, if not identical with, *Irpex tulipifera* and until the limits of the species are better known the writer hesitates to refer the above specimen with certainty.

darker in mature plants but often remains white, sometimes assuming an ochraceous tint in herbarium specimens.

Of the above-named species, the first three have been referred to *P. adustus* by Murrill. *P. adustus* was described by Willdenow[1] in 1787. *P. crispus* was first described as a species by Persoon,[2] in 1799, and was later (1815) accepted by Fries[3] and so maintained by him in his 'Hymenomycetes Europaei.' *P. Burtii* was described from Vermont by Peck,[4] in 1897, and has not since been reported. *P. fumosus* was first described by Persoon,[5] in 1801, and *P. fragrans* by Peck,[6] in 1878. There are several other names for plants closely related to, if not identical with, these species but the writer has had no opportunity to study them. One of these, *P. subcinereus,* described by Berkeley, in 1839, is said to have been repudiated by its author and the plants referred to *P. adustus. P. Halesiae* Berk. & Curt.[7] is probably distinct, and *P. Lindheimeri* Berk. & Curt.[8] is not at all related to *P. adustus,* as stated by Murrill, but is a large-pored species with a brown context.

In working over the collections referred to *P. adustus* in the herbarium of the Missouri Botanical Garden, the herbarium of Dr. E. A. Burt, and the writer's herbarium, it became evident that we are here concerned with a species that has been used as a sort of dumping-ground for all plants with a black hymenium and a rather thin context, while plants of thicker context and lighter-colored hymenium have been referred to *P. fragrans* or to *P. fumosus,* according to whether a pleasant odor was or was not noticed in the plants. Such procedure has resulted in the bringing together of a heterogeneous mass of material under the name *P. adustus.* This material was very readily separated into three fairly distinct sections besides the collections that properly belonged under

[1] Fl. Berol. p. 392. 1787.
[2] Persoon, C. H. Obs. Myc. 2: p. 8. 1799.
[3] Fries, E. Obs. Myc. 1: p. 127. 1815.
[4] Bul. Tor. Bot. Club 24: p. 146. 1897.
[5] Syn. Fung. p. 530. 1801.
[6] Rept. N. Y. State Mus. 30: p. 45. 1878.
[7] Grev. 1: p. 52. 1872.
[8] *Ibid.* p. 50. 1872.

P. fumosus. After considerable study the writer has decided that to *P. adustus* should be referred those collections with a thin, finely tomentose pileus, a thin, even margin, and minute black pores. The species does not grow densely imbricate as in *P. crispus* (see pl. 23 fig. 7) and does not have the crisped margin of that species. The illustration of *P. adustus* given by Patouillard[1] represents our plant very well. From *P. Burtii* it is to be distinguished by the smaller and more equal pores, the thinner, sterile margin of the pileus, and the firmer context. It is much more abundant than the other three species and frequently grows semi-resupinate.

According to Fries, *P. crispus* differs from *P. adustus* in having a thin, crisped, margin and large unequal pores. One lot of segregates from my *P. adustus* material possesses just those distinguishing characters, and I have, therefore, revived the Friesian name and applied it to my plants. They are certainly distinct from the specimens referred to *P. adustus* though connected by intergrading forms to some extent. The illustrations (pl. 23 figs. 7 and 8) show typical specimens of the two species.

I have seen no specimens other than the types that could be referred to *P. Burtii.* The type specimens differ from the above conception of *P. adustus* in having a somewhat thicker context, a thicker margin that is fertile below, and larger and more unequal pores. The hymenium is black, as in that species, and the surface of the pileus is finely tomentose. The flesh of the pileus is also very soft and almost floccose in texture. It has been held by some that the mouths of the tubes in *P. adustus* become larger and more irregular in mature plants, and if such a character stood alone in the differentiation of these forms it probably should not be considered a specific character. But it is the writer's opinion that in *P. adustus* they do not become much larger in old plants, and since *P. Burtii* differs also from that species in the other characters mentioned above, we must consider it a valid species, at least until other collections throw more light on the subject. From *P. crispus* it may be separated by the fact that the

[1] Tab. Anal. Fung. *f. 142.*

margin is not crisped, sterile, and thin, that the pubescence of the pileus is not nearly so prominent, and that the context is soft and floccose. The type specimens are not densely imbricate as in *P. crispus* but more nearly approach the condition found in *P. adustus.*

The microscopic characters of these three species are identical and do not afford additional means of separating them. The tramal tissue of the pores is decidedly brown in color, the hyphae are small, and a large percentage of them are cut transversely in a cross-section of the hymenium. The spores in all three species are oblong or oblong-ellipsoid, and measure 3.5–4.5 × 1.5–2.5 μ. There are no cystidia or other sterile bodies in the hymenium.

In endeavoring to find characters on which to separate the three above-named species (and especially *P. adustus*) from specimens heretofore referred to *P. fumosus* and *P. fragrans,* recourse was had to microscopic sections of the hymenium. It was at once apparent that when longitudinal sections were prepared, according to directions given on page 678 of this paper, the tramal tissue of the tubes of *P. adustus, P. crispus,* and *P. Burtii* were decidedly brown in color, while those of *P. fumosus* and *P. fragrans* were entirely hyaline, except for the eosin stain. This character has been tested out thoroughly and is believed to be a satisfactory and constant one on which to differentiate these two groups of species. By obscuring the labels on the slides containing the sections of the different species it was found possible to easily separate the sections of the species of the one group from those of the other group by this character, and then verify the separation by uncovering the labels. Since suitable sections can be readily prepared in a very few minutes, the task of deciding between the two groups is an easy one when they cannot be readily separated on the general appearance of the specimens. Some such method of procedure is especially desirable in separating *P. adustus* from *P. fumosus,* since thin or young specimens of the latter are easily confused with the former species. However, care must be taken not to confuse the dark color sometimes obtained in thick sections of *P. fumosus* with the truly

brown color of the hyphae in *P. adustus*. In the hyphae of the latter species the color is brown, whether the sections are thick or thin. This test will usually apply to cross-sections of the tubes as well as to longitudinal sections, except that when the hymenium of a growing specimen is bruised, dried, and then sectioned, the mouths of the tubes and the hyphae at the ends of the tubes often show a brownish discoloration that may be confusing. *P. crispus* and *P. Burtii* usually are easily distinguished without this test, but the results are even more marked in the case of those two species than in *P. adustus*.

When Peck first described *P. fragrans* he stated that it was closely related to *P. fumosus,* but differed in having unequal pores and an agreeable odor. In a later report he remarked that it should perhaps be considered a variety of that species. Microscopically the two plants are the same. There are no cystidia and the spores are oblong-ellipsoid, and measure 4.5–6 × 2–3 μ, thus being slightly larger than the spores of the three species discussed above. The spore characters given for both species in the 'North American Flora' are erroneous. From our present knowledge of the variability of odors in the fungi[1] we are not warranted in laying much stress on the fragrant odor ascribed to *P. fragrans*. Bresadola[2] discusses *P. fumosus* under the name *P. imberbis* and states that the plant at times has a subanise odor. I have never obtained such an odor from plants heretofore referred to that species, but frequently the plants do have an odor that I would not describe as pleasant. In the face of such evidence, it seems reasonable to conclude that the odor alone should not separate the two species in question. As to the size and regularity of the pores of the two species, I find collections of *P. fumosus* in which the younger specimens have minute pores and the older ones have large and irregular pores, and collections of *P. fragrans* with both large and small pores. I conclude,

[1] e. g., *Polyporus graveolens* Schw. I have collected this species several times and have had growing plants under observation for three seasons and at no time have I been able to obtain the slightest trace of an odor that would warrant the application of "sweet knot" to that species. Similar results have been reported by others. There is good authority, however, for stating that it is at times very fragrant.

[2] Fung. Trid. p. 29.

therefore, that we are here dealing with a character that varies with the age of the plants or even varies in different plants of approximately the same age. In other characters the two species are identical. Bearing in mind then the following points: (1) Peck's admission concerning his species, (2) the little reliance that is to be placed on odors in at least some of the fungi, (3) the evidence that *P. fumosus* is sometimes fragrant as it grows in Europe, and (4) the variability in the size of the pores in a single collection, we can only conclude that *P. fragrans* is at most only a form of *P. fumosus* and not worthy of a distinct name.

There are a few other names that need to be mentioned before dismissing this group of species. *P. salignus* Pers. ex Fries is generally held to be *P. fumosus,* and Fries' illustration[1] certainly agrees with the species as it grows in this country. *P. Holmiensis* Fries, as distributed by Romell,[2] is surely our plant and it is so regarded by Bresadola. *P. imberbis* Bull. ex Fries, as represented by Bresadola, is the same plant, but the name was not recognized by Fries in his 'Systema Mycologici' and so cannot be used for our plant.

The following key will aid in distinguishing the four species presented here:

Pileus rather thin; hymenium black or smoky black; tramal hyphae distinctly brown in section 1

Pileus thicker; hymenium pallid to somewhat smoky; tramal hyphae hyaline or nearly so in section ..... *4. P. fumosus*

1. Pileus finely tomentose; margin thin, even, sterile below; context firm when dry; pores minute; plants slightly, if at all, imbricate ..*1. P. adustus*

   Pileus adpressedly fibrillose on the margin, usually strigose toward the base; margin thin, crisped or wavy, sterile below; context firm when dry; pores larger and unequal; plants usually closely imbricate ..... *2. P. crispus*

   Pileus finely tomentose; margin acute but thicker than in the preceding species, even, fertile below; context soft and floccose; pores unequal; plants scarcely imbricate ..... *3. P. Burtii*

1. Polyporus adustus Willd. ex Fries. Plate 23, fig. 8.

Pilei *not much imbricate* though somewhat so at times, 1–6 × 3–8 × 0.1–0.6 cm., white to smoky white or pale tan, rarely with reddish blotches or zones, *finely tomentose to short villous-tomentose,* zonate or azonate; margin *thin, even,*

[1] Ic. Hym. 2: *pl. 181.*

[2] Fung. Scand. 11.

often black in dried specimens, sterile below; context white or pallid, firm and corky when dry, 1–4 mm. thick, in large specimens separated from the hymenium by a narrow dark line; tubes less than 2 mm. long, the mouths *grayish black to black, scarcely visible to the naked eye, averaging about 6 to a mm.;* tramal tissue decidedly *brown in color under the microscope;* spores oblong or oblong-ellipsoid, rarely slightly curved, smooth, hyaline, 3.5–5 × 1.5–2.5 μ; cystidia none.

On dead wood of deciduous trees.

Illustrations: Pat. Tab. Anal. Fung. *f. 142.*—Rostk. in Sturm's Deutsch. Fl. **3**: fasc. 16. *pl. 38.*

Specimens examined: Cooke, Fung. Brit. 2.—Ell. N. Am. Fung. 6.—Ell. & Ev. Fung. Col. 206.—Krieg. Fung. Sax. 1319. —Rabenh. Herb. Myc. 412.—Rav. Fung. Am. 421.—Shear, N. Y. Fung. 32.—Mo. Bot. Gard. Herb. 4222 (Newfoundland), 4223 (New York), 3851 (Missouri).—Burt Herb. (collections from Vermont, Ohio, Massachusetts, and New York).—Overholts Herb. 284 (Ohio), 572 (Missouri), 2239 (New York), 1780 (Colorado), and others.

2. Polyporus crispus Pers. ex Fries. Plate 23, fig. 7.

Pilei *more or less densely imbricate and overlapping,* 2–7 × 1–5 × 0.1–0.4 cm., gray to avellaneous, sometimes cinnamon to clay-colored in herbarium specimens, *adpressedly fibrillose toward the margin, usually strigose toward the base,* zonate or azonate; *margin very thin, radiate-lineate, crisped or wavy,* often becoming black, sterile below; context white or pallid, often brownish in herbarium specimens, soft and fibrous to corky, 1–3 mm. thick, usually separated from the hymenium by a narrow dark line; tubes 1–3 mm. long, the mouths *grayish black to black, unequal, irregular, averaging 3–6 to a mm.;* tramal tissue decidedly brown in color under the microscope; spores oblong or oblong-ellipsoid, smooth, hyaline, 3.5–4.5 × 1.5–2.5 μ; cystidia none.

On dead wood of deciduous trees.

Illustrations: Fl. Dan. *pl. 1850.*

Specimens examined: Romell, Fung. Sax. 8 (as *P. adustus*).—Thuem. Myc. Univ. 604 (as *P. fumosus*).—Mo. Bot.

Gard. Herb. 42868, 42848 (Arkansas), 4180 (Missouri).—Overholts Herb. 386 (Indiana), 105 (Ohio).

3. Polyporus Burtii Peck. Plate 23, fig. 4.

Pilei not closely imbricate, 1–2.5 × 2–5 × 0.3–0.5 cm., gray or pinkish buff, *finely tomentose,* azonate; margin *acute but rather thick, deflexed, even, concolorous, fertile below;* context *soft and sub-floccose* in dried plants, 2–4 mm. thick; tubes 1–2 mm. long, *the mouths grayish black to smoky black,* unequal, irregular, *averaging 2–4 to a mm.;* tramal tissue decidedly brown in color under the microscope; spores oblong-ellipsoid, smooth, hyaline, 4–4.5 × 1.5–2 μ; cystidia none.

On stump of yellow birch. Known only from the type locality, Middlebury, Vermont.

Specimens examined: Burt Herb. (type collection).

4. Polyporus fumosus Pers. ex Fries. Plate 23, fig. 3.

Pilei simple or imbricate, 2–10 × 3–15 × *0.5–2 cm.,* white to ochraceous or smoky white, sometimes stained with reddish, *finely tomentose to glabrous, sometimes with a rather broad, marginal furrow;* context white or pallid, soft corky to woody when dry, *2.5–10 mm. thick,* usually zonate, always separated from the hymenium by a narrow dark line, *anise-scented or with a disagreeable odor;* tubes 1.5–4 mm. long, the mouths *white to grayish black, usually becoming black when bruised,* averaging 3–4 to a mm.; tramal tissue hyaline or nearly so under the microscope; spores oblong-ellipsoid, smooth, hyaline, 4.5–6 × 2–3 μ; cystidia none.

On dead wood of deciduous trees, especially elm.

Illustrations: Fries, Ic. Hym. *pl. 181* (as *P. salignus*).—Bres. Fung. Trid. *pl. 135* (as *P. imberbis*).—Massee, Brit. Fung. Fl. *f. 14–15.*—Rostk. in Sturm's Deutsch. Fl. **3**: fasc. 16. *pl. 42.*

Specimens examined: Ell. & Ev. N. Am. Fung. 2902.—Shear, N. Y. Fung. 31.—Thuem. Myc. Univ. 5.—Mo. Bot. Gard. Herb. 43648 (Missouri), 4277 (Kansas).—Overholts Herb. 455, 527 (Ohio), 436 (Canada), 370 (Indiana), and others.

The White Species of Polyporus — Those Watery and Fleshy-Tough when Fresh and with White Context and Spores

This group of plants has probably been the source of more trouble and exasperation to those collecting them than any other group in the *Polyporaceae*. Collectors have sent them to various mycologists for determination, and quite often no two will agree on the name that should be applied to any one form.

The group of species with which we are here concerned has been divided into two genera by Murrill, namely, the genus *Tyromyces* and the genus *Spongipellis*. Since the characters that separate the latter from the former genus are not always well defined, it would seem better had they been united into one genus. The group includes those species found only during the summer and fall, growing on logs or on living trees, and further characterized by being white or whitish throughout, and having a more or less watery and soft fibrous context. Some of the species have characteristic odors that will usually aid in their identification. When dry the context of some of these is soft and friable, sometimes more solid, and sometimes differentiated into an upper soft portion and a lower firm portion. We cannot include here all of the species referred by Murrill to the two above-named genera, partly because there has been no opportunity to study all of them and partly because many of them are limited in their distribution and are only infrequently found by collectors. Those that are of common occurrence in the Ohio and the upper Mississippi River valleys have been studied and the results here presented. The series thus limited includes the following species: *P. albellus* Peck, *P. caesius* Schrad. ex Fries, *P. chioneus* Fries, *P. delectans* Peck, *P. fumidiceps* Atk., *P. galactinus* Berk., *P. lacteus* Fries, and *P. spumeus* Sow. ex Hornemann. These are not all closely related and most of them are not difficult to determine but they have been more or less confused in this country, and their distinguishing characters are here pointed out.

P. chioneus, P. albellus, and P. lacteus.—*P. chioneus* was described by Fries,[1] in 1815. In his 'Hymenomycetes Europaei,' published in 1874 (p. 546), he described it somewhat more fully as follows: "Albus, pileo carnoso, molli, laevigato, azono, postice saepe porrecto, margine inflexo; poris curtis, exiguis, rotundis, aequalibus, integerrimis. . . . . Ad truncos v. c. *Betulae*. . . . . unciam latus, odore acido." In 1878 Peck[2] described *P. albellus* from New York, also growing on birch. Peck evidently was not acquainted with *P. chioneus,* but he regarded his species as probably more closely related to *P. paradoxus* Fries and *P. betulinus* Bull. ex Fries. The only points of difference in the descriptions of *P. albellus* and *P. chioneus* are: (a) in size, Peck's species being described as "two to four inches broad, one to one and a half thick," and (b) in pubescence, the pileus being "smooth or sometimes slightly roughened by a slight strigose tomentum." Both descriptions mention the soft context, white color, and "acid" odor. Saccardo[3] has listed *P. albellus* as a synonym for *P. betulinus,* and while the general form and size of the two species is at times somewhat similar, it does not require close observation to distinguish them. The same cannot be said of *P. albellus* and *P. chioneus.* Murrill[4] has listed them as synonyms and the writer has expressed the same opinion in a recent paper.[5]

P. lacteus may well be brought into the discussion at this point. It was described in 1821. The description and figure[6] call for a plant similar in size and habit to *P. chioneus* but differing from that species and from *P. albellus* in having a decidedly pubescent pileus and a lacerated and labyrinthiform hymenium. These characters should be sufficient to separate at once *P. lacteus* from the other two species, and the writer can neither accept nor understand the determinations of those who would refer our common plant with a glabrous pileus and

[1] Obs. Myc. 1: p. 125. 1815.
[2] Rept. N. Y. State Mus. 30: p. 45. 1878.
[3] Syll. Fung. 6: p. 139. 1888.
[4] N. Am. Fl. 9: p. 35. 1908.
[5] Ann. Mo. Bot. Gard. 1: p. 97. 1914.
[6] Fries, E. Ic. Hym. 2: *pl. 182. f. 1.*

even hymenium to *P. lacteus*. Romell,[1] after a short description of *P. lacteus* as he understands it, says:

> "This species seems to be identical with one known in America as *Polyporus chioneus*. . . . . My specimens agree with the authentic specimens of *P. lacteus* at Kew. In Fries' herbarium neither *P. lacteus* nor *P. chioneus* is represented by authentic specimens as far as I know. There is, however, a collection referred to *P. chioneus* by Robert Fries, and this collection differs from my plant not only by the *glabrous surface of the pileus* but also by having the hyphae substantially *parallel and simple*. . . . . ." (Italics are the writer's.)

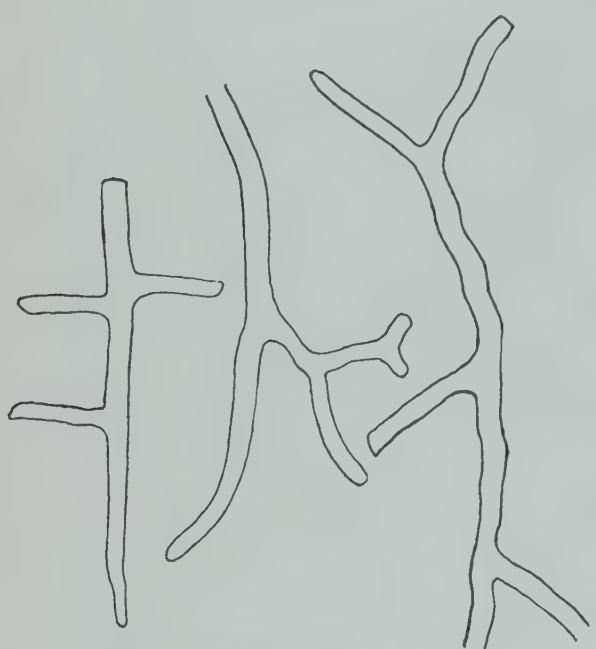


Fig. 3. Hyphae of *P. chioneus*.

It is unfortunate if, with the easy access to Fries' description, American mycologists of repute have sent specimens of a pubescent *Polyporus* to Europe under the name, *P. chioneus*. On the other hand, if the determination were that of an amateur it should not have been seriously considered by Mr. Romell. Whichever may have been the case, it is the writer's opinion that such determinations are the exceptional ones and not the rule, for the plant that is usually referred to *P. chioneus* (including *P. albellus*) is usually, if not always, entirely glabrous and has even tube mouths. In fact, it is the writer's opinion that *P. lacteus* and *P. chioneus* have been less confused in this country than in Europe. If there has been a tendency to confuse *P. lacteus* with anything it is with *P. galactinus*, as I have found several collections so misdetermined. The important point of the extract from Romell's paper is, however, that the collection to which reference is there made as having a glabrous pileus and simple hyphae in the context, in all probability represents the species that is interpreted in this paper as *P. albellus*.

Having fixed upon the distinguishing characters of *P.*

[1] Hym. Lapp. p. 15.

chioneus and accepting Fries' idea of *P. lacteus,* it becomes an easy matter to differentiate between *P. chioneus* and *P. albellus.* As stated above, and as will be seen in the accompanying illustration (fig. 4), the hyphae in the context of *P. albellus* are unbranched or at most very infrequently branched, while those of *P. chioneus* (fig. 3) are branched to a very great degree, and they vary considerably in size, some being narrow (5–6 μ) and others twice as thick. This is not the only distinguishing character, nor the one that was first hit upon by the writer, although it is probably the most reliable. The relative thinness of the pileus in proportion to its length is a distinguishing character of *P. chioneus.* In other words, the pileus is usually thin and spreading in *P. chioneus,* while in *P. albellus* it is thicker, convex or ungulate, and triangular in section. This is only a general statement of a character that varies considerably. An additional character is found in an examination of a cross-section of the hymenium, though the sections must be cut very thin to see it at its best. In sections of *P. albellus* the hyphae in the trama of the pores appear to run in all directions and give a peculiar, ever-changing appearance as they are viewed at changing foci. They are also all of one size. In *P. chioneus* the hyphae in the trama of the pores all run in one direction and practically all are cut transversely in a cross-section of the hymenium. The trama is seen to be made up of a background of a pseudocellular structure, with minute openings that indicate the cavities of the closely compacted hyphae. Interspersed over this background one sees cross-sections of hyphae two to three times larger, and standing out much more plainly than the sections of the compact hyphae in the background. It was at first thought these

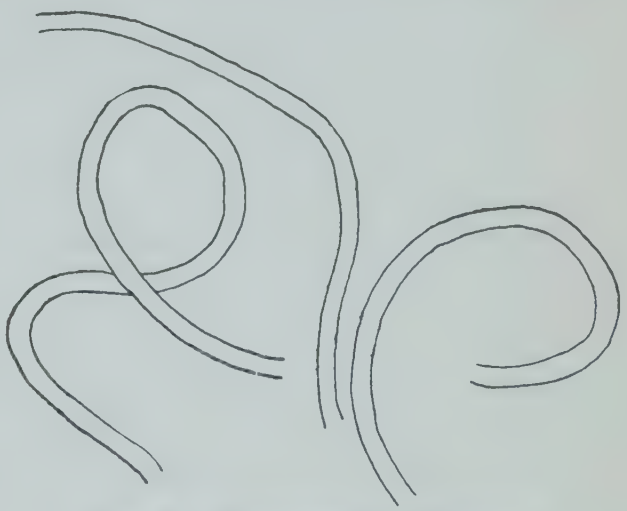


Fig. 4. Hyphae of *P. albellus.*

larger hyphae might belong to some other fungus living within the tissues of this species. This supposition is rendered improbable, however, by the fact that they are invariably present in all collections, and that while other fungi frequently attack all of these white species, their hyphae are invariably much smaller than those of the fungi attacked.

The evidence seems very clear, however, that these two species should be considered as distinct. When once differentiated they can usually be separated on the basis of their general habit, without recourse to the character of the branched or unbranched hyphae in the context, though that character can always be relied upon in establishing beyond a doubt the identity of the species. In other characters the two species are very similar. Both are glabrous or practically so; are covered with a thin grayish or yellowish pellicle that becomes more evident when the plants are dried; have a sweet acid odor when fresh, a soft and friable context when dry; and the spores are the same, being cylindric, often slightly curved, and measuring 3–4×0.7–1.5 μ. There are no cystidia.

There is considerable doubt in the writer's mind as to whether the true *P. lacteus* occurs in this country. There is a collection in the herbarium of the Missouri Botanical Garden and another in the writer's herbarium that should perhaps be referred to that species, but the hymenium has been disorganized by the growth upon it of another fungus, so that no spores are present. If future collections should show that the spores are similar to those of *P. chioneus,* the plants should in all probability be referred to *P. lacteus.* The pileus is somewhat strigose or fibrillose-pubescent, though the mouths of the tubes are not labyrinthiform. The pileus is too pubescent for either *P. chioneus* or *P. albellus* to which latter species the plants were once referred by Lloyd. It is possible that they represent *P. lacteus* as more recently defined by Lloyd.[1] I have seen no specimens so referred by him and his description of the plant as "a common white species" and again as "a frequent plant" throws some doubt on my opinion, for the plant is a rare one.

[1] Letter No. 49, p. 14.

According to the writer's notes on specimens of *P. lacteus* in the herbarium of the New York Botanical Garden, that species, as it appeared in the 'North American Flora,' is *P. albellus* as here defined, at least in part. Neither can the writer accept Romell's interpretation of *P. lacteus,* but if such a plant exists it must agree in the main with Fries' description and figure, and neither of the above interpretations do so agree. I do not know what Bresadola's latest ideas on the subject are, but at one time he regarded *P. lacteus* and *P. chioneus* as synonyms—a position just as untenable as that taken by Murrill and Romell.

According to the above interpretation of *P. chioneus* and *P. albellus,* the presentation of the two species in a recent paper[1] by the writer should be modified, and those collections that show simple hyphae in the context should be referred to *P. albellus* and those with branched hyphae should be referred to *P. chioneus.*

P. delectans and P. spumeus.—The first one of these species was described by Peck,[2] in 1884, from specimens collected in Ohio by Morgan. It is a large or medium-sized plant and was described as having a fleshy-fibrous context, a glabrous or floccose-tomentose pileus, and long tubes with large unequal mouths. By this last character and by the large size of the plant and the ellipsoid or subglobose spores it is easily distinguished from the species discussed above. In size of pores and length of tubes it is intermediate between the above species and *P. obtusus* Berk. A much more closely related species, however, is *P. spumeus.* The original notes of Sowerby on this species are very meager. The plant is described as "oozes from decaying elms in a very soft frothy mass, hardening in a day or two; and if it dries favorably, the pileus becomes hispid. The pores are small and nearly round; the tubes not long." In Sowerby's text[3] this species is followed by *P. betulinus.* Plates 211 and 212 are cited as representing the two species, respectively. Plate 211 shows a

[1] *loc. cit.* p. 97.
[2] Bul. Tor. Bot. Club 11: p. 26. 1884.
[3] Colored Figs. Eng. Fung. *pl. 211–212.* 1797–1803.

plant with a substipitate base, an incurved margin, and short tubes. One figure shows the plant from a front-underneath view, the other shows half of the plant with the cut surface outward and the hymenium upward. Plate 212 shows practically the same thing but with a little more detail, and it is a fair representation of *P. betulinus*. All later descriptions of *P. spumeus* are either based entirely on pl. 211, or else on plants that have no resemblance to the one that has since been referred to *P. spumeus*. Fries' description[1] says: "basi stipitiformi, margine incurvato."

This gives us but two alternatives from which to choose. Either Sowerby confused his illustrations of *P. spumeus* and *P. betulinus* and inserted two plates of the same species (*P. betulinus*), or else there existed at that time a plant closely related to *P. betulinus* but growing on elm and thought by Sowerby to be distinct. Since the mutual resemblance of Sowerby's two plates is so great, it is the writer's opinion that he had drawn two plates of *P. betulinus* and by mistake inserted both of them instead of one of that species and one of *P. spumeus*. This theory is borne out by the fact that he makes no mention of a stipe-like base nor an incurved margin to the plant. We may also conclude that Fries' description was drawn, in part at least, from pl. 211, for it is inconceivable that with access to Sowerby's figure he would have referred to that species a plant that departs so widely from the authentic illustration, unless he was also of the opinion that pl. 211 was a mistake.

This mistake (for so it seems we must regard it) has caused some little confusion in the literature. Fries' idea of *P. spumeus* was evidently gained, in part at least, from Sowerby's plate, for he refers as a synonym for *P. spumeus, Boletus suberosus* of Wahlenberg[2]. But Wahlenberg was aware of the existence of a *Boletus suberosus* of Linnaeus[3] and expressed the doubt that his species was the same as that one. *Boletus suberosus* of Linnaeus has always been regarded as

[1] Hym. Eur. p. 552. 1874.
[2] Fl. Upsal. p. 457. 1820.
[3] Sp. Plant. p. 1176. 1753.

a synonym for *P. betulinus*. In 1823 Hornemann[1] published a figure of *P. spumeus* entirely different from Sowerby's original figure, but in all probability a better representation of his original species. It was not, however, so accepted at the time. In the text accompanying the plates in 'Flora Danica,' Hornemann refers to Sowerby's original figure as a variety (var. *stipitatus*) of *P. spumeus*. This was evidently only a makeshift to dispose of a troublesome figure, and since the figure itself was evidently an error, Hornemann's disposition of it need have no weight. Subsequent writers did not concur in his opinion, however, and the confusion was only made worse, for now some regarded that there were two distinct plants passing under the name of *P. spumeus*. In Hooker's 'English Flora,'[2] in which the fungi were written up by Berkeley, both Hornemann's and Sowerby's illustrations are cited as representing *P. spumeus*, and Hornemann's figure is given priority in the order of citation. Again the plant is described as possessing an obsolete stipe and an incurved margin—characters either taken from Sowerby's illustration or copied from Fries. That Berkeley was in doubt as to the correctness of Sowerby's plate is evidenced by the statement: "According to Fries, the figure of Sowerby represents the species in an imperfect state. . . . . ." In 1874 Fries[3] accepted Sowerby's figure as representing *P. spumeus* and referred Hornemann's figure to *P. epileucus*. This reference was evidently followed by Saccardo. Berkeley[4] published an illustration of *P. spumeus* that corresponds well with Hornemann's figure and agrees with the plants since referred to that species. Thus there has arisen an interesting situation in which, according to the writer's interpretation, a well-known species is referred to an erroneous illustration that cannot possibly represent it, while the authentic illustratration is referred to another species. Of course it is possible that Hornemann may have misinterpreted Sowerby's *P.*

[1] Fl. Dan. *pl. 1794*. 1823.
[2] Eng. Fl. 5²: p. 139. 1836.
[3] Hym. Eur. p. 552. 1874.
[4] Outl. Brit. Fung. *pl. 16. f. 4*. 1860.

spumeus, in which case the name should be written *P. spumeus* Hornemann, Fl. Dan. *pl. 1794.* 1823, since there is no doubt that Hornemann's figure represents *P. spumeus* as it is known in Europe to-day. But the writer prefers to accept Hornemann's plate as a correct interpretation of Sowerby's species (disregarding pl. 211) and write the name as *P. spumeus* Sow. ex Hornemann. If the writer's theory is correct, there never existed a plant, the name of which could be written as *P. spumeus* Sow. ex. Fries, Syst. Myc. 1: 358. 1821,[1] since Fries never illustrated the plant, and his descriptions, several times repeated, were based, in part at least, on the erroneous pl. 211 of Sowerby.

In the American literature the plant was first described by the writer in a recent paper.[2] The relation of Sowerby's figure to the species was not then understood and the statement was there made that "the plants so referred do not agree with the figure given by Sowerby, nor with Fries' description." There are but few references to its occurrence in this country, although it is a fairly common species. Lloyd reports receiving it from several widely separated localities.

Whether others may agree with the writer or not, the evidence here presented should at least have the effect of doing away with the inconsistency of citing both Sowerby's illustration and that of Hornemann as representing the same species.

P. spumeus is not likely to be confused with any species except *P. delectans.* These two intergrade to some extent. The former species has a strigose-tomentose surface to the pileus while the latter is glabrous or only slightly tomentose. Heavy rains or a little handling of the plant may cause the pubescence on *P. spumeus* to become matted and appressed, but when specimens are found growing imbricated so that the lower pilei are protected by the ones above, the character is very marked. The tubes in both species are long and slender, but in *P. delectans* the mouths are larger and more sinuous, usually measuring 0.5–1 mm. in diameter, while those of *P. spumeus* are smaller, measuring about 3–4 to a mm., and col-

[1] cf. Ann. Mo. Bot. Gard. 1: p. 99. 1914.
[2] *loc. cit.*

lapse when dry. This collapsing is due to the thinness of the dissepiments—a character easily made out in transverse sections of the hymenium. The illustration (pl. 24 fig. 14) shows the larger tubes of *P. delectans*. The spores of the two species are practically the same, varying from ellipsoid to ovoid or subglobose, and measuring 5–6×4–5 μ. They are frequently guttulate in both species. There are no cystidia in the hymenium.

P. galactinus.—This species is a fairly well-marked one and only its distinguishing features will be pointed out here. It was originally described by Berkeley from specimens collected in Ohio by Lea. It is eastern in its range in the United States, occurring from Maine to Missouri and probably no farther south than West Virginia. There are but three common plants in this section of *Polyporus* that possess characteristic odors when fresh and growing. *P. galactinus* is one of them. The odor is usually described as "acid," but to the writer it is a very pleasant and fragrant odor, but not persisting in the dried plants. Characters are not wanting to separate this species from the group just discussed in this section. The pileus is strigose-pubescent, as shown in the illustration (pl. 24 fig. 15), the tubes are very small, and the spores are minute, ellipsoid or subglobose, uninucleate, and measure 3–4 × 2–3 μ. From *P. delectans* and *P. spumeus* it may be separated by the minute pores and the smaller spores. From *P. fumidiceps* Atk. it differs in the decidedly pubescent pileus and larger size. From *P. caesius,* which it resembles in its hairy covering, it differs in its larger size and ellipsoid spores. There are no cystidia.

P. caesius.—This species has long been recognized as a well-marked one, characterized by the villous-strigose pubescence on the pileus, the bluish or grayish blue tint often present on the hymenium, and the minute, cylindric, curved spores. From *P. galactinus* it is separated by its small size and different spores; from *P. chioneus* and *P. albellus* by the pubescent pileus; from *P. lacteus* by the more strigose pileus and the unbranched hyphae of the context.

P. fumidiceps.—This species was described by Atkinson[1] in 1908, and has not since been reported. Since the writer finds it to be a rather common species in Missouri, and since a description has not appeared in the American literature, a few notes will be appended and the plant described on a following page.

In size and shape the species corresponds most closely to *P. chioneus,* but it is of a different color and the spores are ellipsoid to subglobose. From *P. galactinus* and *P. caesius* it is separated by the almost or quite glabrous pileus and from the latter also by the spores. The writer finds it most often on dead willow logs in willow thickets along river bottoms. The types were described from similar locations. Fresh plants have the same peculiar fragrant odor that is found in *P. galactinus.*

The following key will aid in the determination of the species here discussed:

Spores cylindric-oblong, often allantoid 1
Spores ellipsoid to globose 3
1. Pileus villous-strigose; hymenium often bluish or grayish blue ..*5. P. caesius*
   Pileus glabrous or very slightly pubescent 2
2. Hyphae of context simple or very slightly branched; pileus usually triangular in section; tubes usually 4–9 mm. long .....*2. P. albellus*
   Hyphae of context much branched; pileus usually more applanate; tubes 1–3 mm. long .....*1. P. chioneus*
3. Spores 5–6 μ in longest direction; plants not fragrant when fresh 4
   Spores 2–4 μ in longest direction; plants fragrant when fresh 5
4. Pileus strigose-tomentose or strigose-hispid, especially on the margin; tubes collapsing on drying, the mouths equal, small, averaging 3–4 to a mm. .....*3. P. spumeus*
   Pileus glabrous or floccose-tomentose; tubes scarcely collapsing on drying, the mouths usually somewhat sinuous, averaging 1–2 to a mm. .....*4. P. delectans*
5. Pileus glabrous or nearly so .....*7. P. fumidiceps*
   Pileus conspicuously pubescent, often strigose-tomentose at the base .....*6. P. galactinus*

1. Polyporus chioneus Fries. Plate 24, fig. 13, 16b

Pileus soft and watery when fresh, rigid when dry, 2–7 × 1–6 × *0.5–1.5* cm., white, often grayish or yellowish when dry, glabrous or nearly so, *covered with a thin continuous gray or yellowish pellicle that becomes more evident when the plants are dried;* context white, *usually with a fragrant*

[1] Ann. Myc. 6: p. 61. 1908.

odor when fresh, soft and friable when dry, *2–7 mm. thick; tubes 1.5–3 mm. long,* the mouths white or yellowish, averaging 3–4 to a mm.; spores cylindric or allantoid, minute, hyaline, 3–4 × 0.7–1.5 μ; cystidia none; hyphae of context hyaline, much branched.

On dead wood of deciduous trees.

Specimens examined: Mo. Bot. Herb. 4311 (Missouri).—Burt Herb. (collections from Vermont and New York).—Overholts Herb. 2325, 2261, 2277, 2276 (New York), 2326 (Ohio).

2. Polyporus albellus Peck.

Plate 23, fig. 5, Plate 24, fig. 16a.

Pileus soft and watery when fresh, rigid when dry, *more or less triangular in section,* 1–8 × 1–7 × *1–4* cm., white or yellowish, glabrous or nearly so, *covered with a thin yellowish pellicle that is more evident in dried plants,* but often disappears in patches; context white, soft and friable when dry, *0.5–3 cm. thick;* tubes 4–9 mm. long, the mouths white or yellowish, averaging 3–4 to a mm.; spores cylindric or allantoid, minute, hyaline, 3–4 × 0.7–1.5 μ; cystidia none; hyphae of context hyaline, unbranched or nearly so.

On dead wood of deciduous trees.

Specimens examined: Mo. Bot. Gard. Herb. 43756 (Idaho).—Burt Herb. (collection from Pennsylvania).—Overholts Herb. 591 (Vermont), 408, 149, 207 (Ohio), 2243, 2270 (New York), 440 (Missouri).

3. Polyporus spumeus Sow. ex Hornemann.

Plate 24, figs. 10, 11, 14a.

Pileus soft and watery when fresh, rigid on drying, *5–20* × *6–20* × *2–6 cm.* (much thinner when dried), white or somewhat yellowish, *villous-strigose or matted strigose-tomentose;* context white, rigid on drying, 1–3 cm. thick; *tubes 0.5–1.5 cm. long,* collapsing when dried, the mouths white or yellowish, *averaging 2–4 to a mm.;* spores *ellipsoid to subglobose,* hyaline, smooth, often once guttulate, *5–6* × *4–5* μ; cystidia none.

Illustrations: Hornemann, in Fl. Dan. *pl. 1794.*—Berk. Outl. Brit. Fung. *pl. 16, f. 4.*

Specimens examined: Cooke, Fung. Brit. 511[1].—Thuem. Myc. Univ. 709[1].—Mo. Bot. Gard. Herb. 43719 (Missouri).—Overholts Herb. 101 (Ohio), 526, 625 (Missouri).

4. Polyporus delectans Peck. Plate 24, fig. 14b.

Pileus soft and watery when fresh, *3–15 × 5–20 × 1.5–5* cm., white, yellowish, or grayish, *glabrous to finely tomentose;* context white, often with a soft upper layer and a more firm lower layer, firm when dry, 0.5–2 cm. thick; tubes 0.5–1.5 cm. long, the mouths white or yellowish, *averaging 1–2 to a mm.; spores ellipsoid to subglobose,* often uninucleate, hyaline, smooth, *4–5 × 5–6* μ; cystidia none.

Growing from wounds of living trees and on old logs.

Illustrations: Jour. Cinc. Soc. Nat. Hist. 8: *pl. 1.*

Specimens examined: Overholts Herb. 145, 519, 250, 415, 659, 93, 258, 255 (all from Ohio and Missouri).

5. Polyporus caesius Schrad. ex Fries.

Pileus more or less triangular in outline, rather soft and watery when fresh, 1–5 × 1–4 × 0.5–2 cm., white or grayish, *rarely bluish gray, villous-pubescent or strigose;* context white, 3–10 mm. thick; tubes 3–5 mm. long, white or *grayish blue, large, unequal, averaging 1–3 to a mm., the dissepiments thin, torn and lacerated; spores cylindric or allantoid,* smooth, hyaline, *3–4 × 0.7–1.5* μ; cystidia none.

On dead wood of deciduous trees.

Illustrations: Sow. Col. Fig. Eng. Fung. *pl. 226* (as *Boletus albidus*).—Gill. Champ. Fr. *pl. 458.*

Specimens examined: Krieg. Fung. Sax. 1913.—Mo. Bot. Gard. Herb. 43650 (Missouri).—Burt Herb. (collections from Canada and New York).—Overholts Herb. 627 (Missouri), 2271 (New York).

[1] These specimens or sections of specimens are not well preserved. They contain no spores, and while the general appearance, i. e., shape of pileus, size of pores, length of tubes in comparison with thickness of context, etc., are very much the same, the context appears to be more woody and zonate than in our specimens. Ellis N. Am. Fung. 1103 is referred to *P. spumeus* Fries. It is the same as distributed by Cooke, Fung. Brit. 603, under the name *P. spumosus* Fries. There is no such species listed by Saccardo. Lloyd (Letter No. 52, p. 25) refers the Ellis specimen to *Fomes geotropus* Cooke.

6. Polyporus galactinus Berk.

Plate 24, figs. 12, 15, 17.

Pileus more or less triangular in sections, sometimes gibbous behind, rather firm but watery, 3–8 × 5–10 × 1–3 cm., white or yellowish, *strigose-tomentose at the base, short tomentose on the margin;* context fibrous when fresh, hard and *sometimes resinous when dry,* white, 0.3–2 cm. thick, *strongly zonate, with a strong fragrant odor in fresh specimens;* tubes 5–10 mm. long, the mouths white or yellowish, *minute, averaging 4–6 to a mm.;* spores *ellipsoid,* smooth, hyaline, once guttulate, minute, *3–4 × 2–3 μ*; cystidia none.

On old logs in woods, especially in overflow river bottoms.

Specimens examined: Mo. Bot. Gard. Herb. 4092, 43636 (Missouri), 4138.—Overholts Herb. 42, 489, 382, 134, 252, 2178, 511, 611, 583 (mostly from Ohio and Missouri).

7. Polyporus fumidiceps Atkinson. Plate 23, fig. 6.

Pileus *thin,* soft and watery when fresh, 1–4 ×2–5 × *0.5–1* cm., vinaceous buff to avellaneous or wood-brown, *minutely pubescent or glabrous;* context white, watery, *with a strong fragrant odor, 2–5 mm. thick;* tubes 2–5 mm. long, sometimes olive-green within on drying, the mouths concolorous, *averaging 4–5 to a mm.;* spores *ellipsoid to subglobose,* smooth, hyaline, *2.5–3.5 × 1.5–2.5 μ*; cystidia none.

On dead wood of deciduous trees, especially willows, in woods and along overflow river bottoms.

Specimens examined: Mo. Bot. Gard. Herb. 43712 (Missouri).—Burt Herb. (part of type collection, from New York).—Overholts Herb. 552, 2305, 2318 (Missouri).

Polyporus lucidus Leyss. ex Fries, P. Tsugae Murr., P. Curtisii Berk., and Closely Related Species

These species form a rather natural group of plants possessing the common character of a laccate or varnished pileus. *P. lucidus* was described in 1780 by Leysser (as *Boletus*) from plants collected in England. The description calls for a plant with a lateral stipe and it is so figured by English mycolo-

gists. *P. Curtisii* was described by Berkeley, in 1849,[1] from plants collected in South Carolina by Curtis. *P. Tsugae* was more recently described by Murrill[2] from plants collected in New York City on decaying trunks and stumps of *Tsuga canadensis*. *Ganoderma sessile* was described at the same time and by the same author.

In Murrill's first treatment of this section[3] *Polyporus lucidus* was reported as a synonym for *P. pseudoboletus,* the latter name being used for the plant. The species was reported as occurring in most of the states east of the Mississippi River with the exception of the New England states. *P. Curtisii* was there listed as a synonym for *P. pseudoboletus* with the remark that specimens referred to *P. Curtisii* were only variations of the other species, due to age, rapidity of growth, and perhaps to differences in the host. The next species described was *Ganoderma sessile* and that was described as differing from *G. pseudoboletus* in being annual and sessile, with a very acute margin and a more rugose surface. It was reported as occurring in Indiana, New York, Ohio, Alabama, Louisiana, and Kentucky. In the 'North American Flora,'[4] six years later, the names *Ganoderma pseudoboletus* and *Polyporus lucidus* were both entirely omitted and *P. Curtisii* was restored as a specific name. No comment was made as to why this was done, nor as to what disposition was made of the numerous collections previously referred to *Ganoderma pseudoboletus*. The writer has seen material referred to *G. sessile* by Murrill, and the supposition is that all collections, except those belonging under *Polyporus Curtisii,* were referred to his new species *Ganoderma sessile.* This supposition is borne out by the fact that the description of that species is there so amended as to include stipitate forms also, while the species as originally described was limited to sessile forms. We must also conclude that *G. sessile* was regarded by its author as distinct from *Polyporus lucidus* of Europe, else that name or an older one would have

[1] Lond. Jour. Bot. and Kew Gard. Misc. 1: p. 101. 1849.
[2] Bul. Tor. Bot. Club 29: p. 601. 1902.
[3] *loc. cit.*
[4] N. Am. Fl. 9: p. 120. 1908.

been used. Mr. Murrill remarks concerning *Ganoderma sessile*[1]: "Very similar in its stipitate forms to *Polyporus lucidus* of Europe." The American plants are usually referred to *P. lucidus* by European mycologists, and taking into account the general agreement with the European descriptions and illustrations, and the fact that Murrill has consistently failed to cite any distinguishing characters upon which the legitimacy of his species might be established, we must conclude that there is no such distinction to be made between the European and the American plants. The American plant is variable in respect to the presence or absence of a stipe, and that cannot enter into the discussion.

There is a tendency among mycologists[2] to disregard the *Ganoderma Tsugae* described by Murrill. To the writer this species appears to be a perfectly good one, although it cannot be differentiated on host character alone. A further discussion of this species is reserved for a following paragraph.

In 1908 Atkinson[3] described a species of *Ganoderma* which he called *G. subperforatum*. After an examination of the type specimens the writer referred[4] this species to *Polyporus lucidus*. This leaves us three species of this section of *Polyporus* that are found in the central states. There are no spore characters of sufficient importance or constancy that can be used in separating them. There is a color difference but it probably cannot always be relied upon. The pileus of *P. Tsugae* is shining and mahogany-colored or darker; that of *P. lucidus* is of a lighter red color; and that of *P. Curtisii* is yellowish, at least in mature plants. Moreover, *P. Curtisii* is southern in its distribution, not being found north of the Ohio River; *P. Tsugae* is not reported south of Virginia; and *P. lucidus* is not limited in its north and south distribution in the United States.

[1] Northern Polypores, p. 55. 1914.

[2] cf. Atkinson, Bot. Gaz. **46**: p. 335. 1908. *G. Tsugae* is here listed as a synonym for *G. pseudoboletus* (=*P. lucidus*). Later on the same page it is given varietal rank; also Lloyd (Letter No. 52, p. 27) cites it as a synonym for *Fomes lucidus*.

[3] Bot. Gaz. **46**: p. 337. 1908.

[4] *loc. cit.* p. 123.

A more constant difference that serves to separate *P. Tsugae* is the color of the context. In *P. lucidus* and *P. Curtisii* the context is never pure white, but is usually separated into an upper light-colored and a lower brown layer. This lower layer is more firm than the upper one and often contains horny fibers. In *P. Tsugae* the context is uniform in texture and almost pure white throughout, but often with a very slight tinge of brown next the tubes. Under the microscope this effect is magnified. There are no brown hyphae in the context of *P. Tsugae,* while in the other two species brown hyphae are very pronounced, especially in the layer of context next the tubes. A comparison of the size of the hyphae in the three species is interesting but does not always give conclusive evidence as to the identity of the species. The hyphae of *P. Curtisii* vary from 4 to 6 μ in diameter. Those of *P. lucidus* are more variable. In some cases they cannot be differentiated from those of *P. Curtisii* in point of size, but in some specimens they attain a diameter of 10 μ. Those of *P. Tsugae* often attain a diameter of 15 μ. The difference in the branching of the hyphae of these three species is very striking and is shown in figs. 5 and 6, all drawn to the same scale. Figure 5a represents the hyphae of *P. Curtisii,* which are not extremely branched but can by no means be said to be unbranched. Figure 5b shows the hyphae of *P. lucidus,* and the branching does not differ materially from that of *P. Curtisii.* In both species the large hyphae may extend more than across the field of the high-power microscope and not branch at all in that distance. This condition is never found in the hyphae of *P. Tsugae.* There the hyphae are extremely branched, as shown in fig. 6. The large hyaline

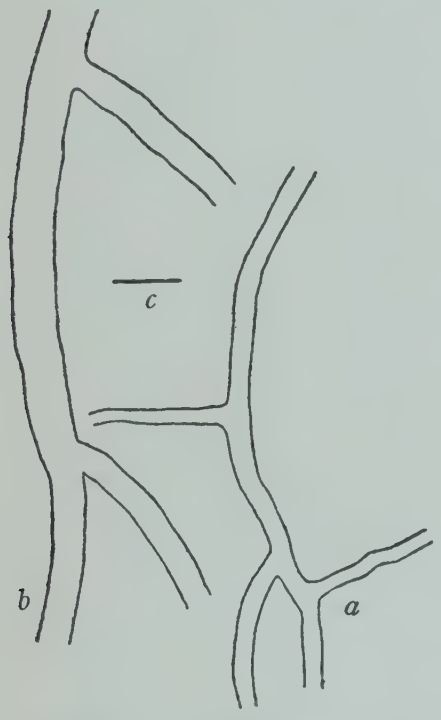


Fig. 5. *a,* hyphae of *P. Curtisii; b,* hyphae of *P. lucidus; c,* width of hyphae of *P. Tsugae.*

hyphae are not continuous for any distance but break up into numerous smaller branches that are often rapidly narrowed to fine thread-like hyphae. This condition must be seen to be best appreciated. It affords, however, another character on which the species can be separated from those closely allied.

The following brief diagnoses of these species is appended:

1. Polyporus Curtisii Berk.

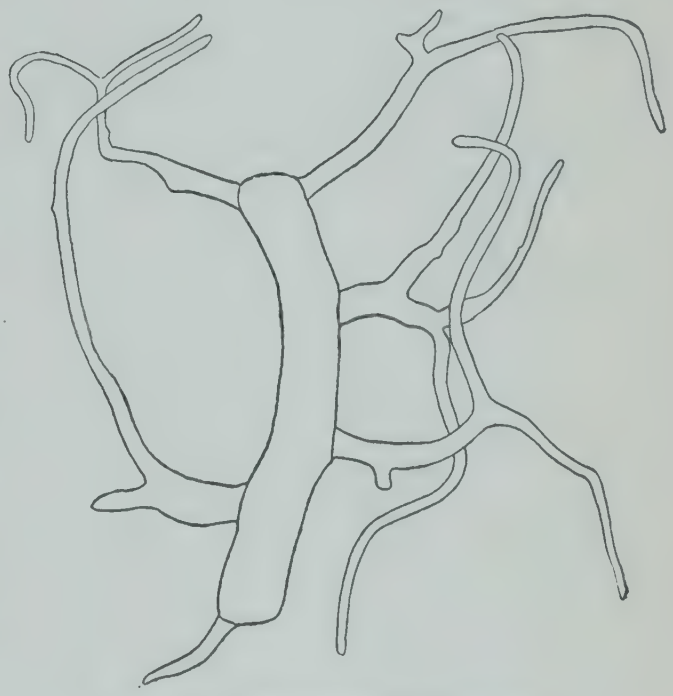


Fig. 6. Hyphae of *P. Tsugae.*

Plants perhaps always stipitate; pileus reniform or flabelliform, 3–12 × 3–20 × 0.7–2 cm., covered with a thin crust that is *at least in part ochraceous in mature plants,* zonate; context *soft and nearly white above, brown and firmer next the tubes,* 0.5–1.5 cm. thick; tubes 0.3–1.2 cm. long, the mouths white to brownish, averaging 3–5 to a mm.; stipe lateral, with color and context as in the pileus; spores light brown, ovoid with a truncate base, apparently echinulate, 8.5–11.5 × 4.5–7 μ; cystidia none; hyphae of context hyaline or brown, *4–6 μ in diameter.*

On and about trunks of *deciduous* trees.

Illustrations: Bot. Gaz. **46**: *f. 1–3.*

Specimens examined: Ell. N. Am. Fung. 802.—Rab.-Wint. Fung. Eur. 3430.—Mo. Bot. Gard. Herb. 1438 (Louisiana), 4746 (Alabama).—Overholts Herb. 305 (Florida), 962, 518 (Missouri), 2235 (New York). Also reported from most of the other states east of the Mississippi and south of the Ohio Rivers.

2. Polyporus lucidus Leyss. ex Fries.

Plants sessile or stipitate; pileus dimidiate or reniform, 3–12 × 3.5–20 × 0.5–2.5 cm., covered with a thin *reddish or*

chestnut crust, zonate; context white to light brown, *usually separated into an upper light-colored layer and a lower brown layer, never entirely white,* 0.2–1.5 cm. thick; tubes 0.3–1.5 cm. long, the mouths white to umber, averaging 3–5 to a mm.; stipe lateral or excentric when present, with color and context as in the pileus; spores light brown, ovoid with a truncate base, smooth or appearing echinulate, 9.5–11 × 5–6.5 μ; cystidia none; hyphae of context hyaline or brown, branched, *4–10 μ in diameter.*

On and about stumps and trunks of *deciduous* trees.

Illustrations: Bot. Gaz. **46**: *f. 5.*—Dufour, Atlas Champ. *pl. 49. f. 116.*—Gill. Champ. Fr. *pl. 457.*—Hard, Mushrooms, *f. 332.*—Krombh. Abbild. u. Beschr. *pl. 4. f. 22–24.*—Rostk. in Sturm's Deutsch. Fl. **3**: fasc. 5. *pl. 13.*

Specimens examined: Ell. N. Am. Fung. 5.—Ell. & Ev. Fung. Col. 202 (Delaware).—Krieg. Fung. Sax. 1116.—Rav. Fung. Am. 5.—Thuem. Myc. Univ. 104.—Mo. Bot. Gard. Herb. 43149, 4095, 4024, 4144 (Missouri), 43939 (Illinois).—Burt Herb. (collection from Vermont).—Overholts Herb. (collections from New York, Florida, Ohio, Illinois, and Missouri).

3. Polyporus Tsugae Murrill ex Overholts n. comb.

Plants stipitate; pileus flabelliform or reniform, 5–15 × 7–20 × 1–4 cm., with a *mahogany-colored or almost black, shining,* incrusted surface, sulcate; context *white or nearly so throughout,* 0.5–2 cm. thick; tubes 0.5–1 cm. long, the mouths white to brown, averaging 4–6 to a mm.; stipe present, with color and context as in the pileus; spores light brown, ovoid with a truncate base, apparently echinulate, 9–11 × 6–7 μ; cystidia none; *hyphae of context very irregular and much branched, up to 15 μ in diameter.*

On or about stumps and trunks of *hemlock and pine.*

Specimens examined: Burt Herb. (collection from Vermont).—Overholts Herb. 2338 (Vermont).

Fomes Ellisianus And. and F. fraxinophilus Peck

Fomes fraxinophilus was described by Peck from New York in 1882. It was first described as a *Polyporus* and later trans-

ferred to the genus *Fomes*. *F. Ellisianus* was described from Montana by Anderson in 1891, and redescribed as *Polyporus circumstans* by Morgan from South Dakota in 1895. The former species is abundant in the central and eastern United States, growing only on the trunks of ash trees. The latter species is found occasionally in the western United States, growing only on trunks of *Shepherdia*.

Lloyd has recently expressed the opinion that these two species are identical, except for host, and he has so treated them in his recent synopsis of the genus *Fomes*. The plants are much alike in their old stages but I cannot agree with him that *Fomes Ellisianus* is *"exactly the same plant"* as our eastern species on the ash. First, there is the distinction in host, but that of itself would not be important. Second, plants of *F. Ellisianus* that are fairly mature have a decidedly corrugated or radiate-rugose surface and a reddish tinge of color. I have seen no indication of either of these characters in *F. fraxinophilus* though I have been familiar with that species for a number of years and have observed it in all stages of growth. When the plants are several years old they become similar in appearance and it would be an easy matter to mistake the one for the other if the host were unknown. But the characters pointed out here are believed to be amply sufficient for retaining the two plants as distinct species.

The following brief descriptions are appended:

1. Fomes Ellisianus Anderson.

Pileus convex to ungulate, 3–10 × 3–8 × 1.5–4 cm., pallid to brown, *radiate-rugose and with a reddish tinge when young, black and usually somewhat rimose with age,* sulcate; context pallid to wood-colored, punky to corky, 0.5–2 cm. thick; tubes 2–6 mm. long each season,[1] *not distinctly stratified,* the mouths white or yellowish, *averaging 2–3 per mm.;* spores oblong-ellipsoid to broadly ellipsoid, 6–8 × 4–5 μ; cystidia none; hyphae hyaline, 3–5 μ.

On Shepherdia in the west-central states.

[1] The tubes in this plant are sometimes continuous to a length of 1.5 cm., but I do not believe that such lengths are attained in a single year's growth.

Illustrations: Bot. Gaz. **16**: *pl. 12.*—Jour. Cinc. Soc. Nat. Hist. **18**: *pl. 1. f. 4* (as *P. circumstans* Morg.).

Specimens examined: Anderson, Paras. Fung. Mont. 537 (as *P. fraxinophilus*).—Baker, Pl. N. N. Mex. 55.—Mo. Bot. Gard. Herb. 4272 (New Mexico).—Burt Herb. (collections from Montana and New Mexico). Also reported from North Dakota and Colorado.

2. Fomes fraxinophilus Peck.

Pileus convex to somewhat ungulate, 2–25 × 3.5–40 × 1.5–10 cm., *at first white,* soon grayish black or black, *not rugose, somewhat rimose* with age, sometimes sulcate; context woody, 0.5–1.5 cm. thick; tubes 2–4 mm. long each season, *indistinctly stratified,* the mouths white to brownish, *averaging 2–3 to a mm.;* spores ellipsoid to ovoid, 5–6 × 6–7 μ; cystidia none; hyphae 3–5 μ.

On living or dead ash trees.

Illustrations: U. S. Dept. Agr., Bur. Pl. Ind. Bul. 32: *pl. 2.*—Hard, Mushrooms, *f. 350.*

Specimens examined: Ell. & Ev. N. Am. Fung. 3302 (Kansas); Fung. Col. 909 (Kansas).—Mo. Bot. Gard. Herb. 4780, 1437, 4826 (Missouri).—Burt Herb. (collections from Kansas).—Overholts Herb. 46, 157, 159, 122, etc. (Ohio), 559, 624 (Missouri), 626 (Iowa). Also reported from Kentucky, Nebraska, Pennsylvania, Indiana, and New York.

Fomes igniarius Linn. ex Gillet and F. nigricans Fries

Much confusion has existed concerning the limits of these two species, and many different ideas are stated in the literature. Murrill has referred *Fomes nigricans* as a synonym for *F. igniarius.* Lloyd has kept them apart, though recognizing a close relationship between them. Others have concluded with Bresadola that we are here dealing with two species that can be easily separated on the presence or absence of setae in the hymenium. Romell has held that such is not the case, but that setae may be present or rare in either species, and has stated that they are usually most abundant near the bottom of the tubes. This would account for the fact that some

observers have stated that they have been unable to find setae in the hymenium of *F. nigricans*.

The original illustration of *F. nigricans* does not agree with any present-day conception of what the species really was. The manner in which the plates for Fries' 'Icones' were gotten together does not at all preclude the existence of grave errors regarding the identity of the species there illustrated. Hence the original illustration of *F. nigricans* has been discounted by careful European workers, they preferring to base the species rather on specimens authenticated by Fries himself. Of these, there appear to be specimens both at Upsala and at Kew.

The *F. nigricans* of my 'Ohio Polyporaceae' proves to be *F. Bakeri* Murrill. The specimens referred by me to *F. igniarius* are of two types. One of these has the pileus convex or ungulate, the surface sometimes becoming rimose, and setae not at all abundant. The second type is most commonly found on birch trees. The pileus is plane or slightly convex, sometimes shining black in color, and the surface often cracks in both directions but does not become roughly rimose. The setae are often more abundant. Of this second form, Lloyd recently wrote as follows concerning a collection sent to him by me: "It agrees with his (Fries') specimens (of *F. nigricans*) both at Upsala and at Kew. . . . . It is usually thinner than typical *F. igniarius* and the setae are more abundant than in the type form."

On the strength of this information I am now able to separate my collections of these forms into what I am convinced are the two species, *F. igniarius* and *F. nigricans,* respectively. I have examined all available material of the two species and have thoroughly confirmed Romell's observation on the presence of the setae. In but one collection was I unable to find setae and I do not doubt that further attempts would show their presence in that instance. It is advisable, however, as stated on a previous page of this article, to cut *longitudinal* sections of the hymenium, since by so doing one will be more likely to strike the setae if there is any variation in their abundance at particular places in the tubes.

The characters cited above do not appear to the writer to be sufficient to warrant the complete separation of the two species. They are sufficiently distinct, however, to enable one to refer to one form or the other all the specimens collected. It has been thought best to refer *F. nigricans* as a variety of *F. igniarius.*

The following diagnosis of the species and its variety is appended:

1. **Fomes igniarius** Linn. ex Fries.

Typical form: Pileus *convex or ungulate,* 3–10 × 5–20 × 2–10 cm., grayish black or black, *rarely roughly rimose with age,* not incrusted; context hard and woody, brown, 0.5–1 cm. thick; tubes 2–5 mm. long each season, the older layers conspicuously white-stuffed or incrusted, the mouths brown, averaging 4–5 per mm.; spores globose or subglobose, smooth, hyaline, 4–6 μ; setae present though sometimes rare, sharp-pointed, 16–25 × 6–8 μ; hyphae 3–4 μ.

Var. *nigricans* Fries: Pileus *plane to convex,* 3–10 × 3–15 × 2–7 cm., black, *sometimes shining black, the surface often cracked in both directions but never roughly rimose;* context and tubes as in the typical form, decidedly white incrusted; spores, setae, and hyphae as above, the setae often abundant.

On trunks of living deciduous trees.

Illustrations: Published illustrations passing under the name of this species and its variety are abundant, but typical representations of my plants so referred are scarce. The type form intergrades into the variety to such an extent that some illustrations are hard to refer. The typical form is represented by Hard, Mushrooms, *f. 349,* and in *pl. 25. f. 18.* of this paper. The variety is well represented by Lloyd, Myc. Notes 29: *f. 193;* Rostkovius in Sturm's Deutsch. Fl. **3**: fasc. 17. *pl. 51.*

Specimens examined[1]: Ell. & Ev. N. Am. Fung. 915 (Kentucky).—Krieg. Fung. Sax. 526.—Thuem. Myc. Univ. 105.—Mo. Bot. Gard. Herb. 4037* (New York), 4043* (New York),

[1] Collections assigned to var. *nigricans* are marked with an asterisk.

43627* (Vermont), 42958 (Florida).—Burt Herb. (collections from Vermont and Canada).—Overholts Herb. 378* (Indiana), 423 (Ohio), 2460* (Vermont), 2256 (New York), 450 (Missouri).

Fomes scutellatus Schw. ex Cooke and F. ohiensis Berk. ex Murrill

These two species are closely related and have on more than one occasion been treated as a single species. *Fomes scutellatus* was first collected by Schweinitz on dead *Syringa* in Pennsylvania. It has since been reported on a few other hosts, namely, alder, witch-hazel, and sweet-gum. *F. ohiensis* was originally described from Ohio by Berkeley and is a very common species in that state. It is especially abundant on dead limbs on the ground in woods in September and October. Quite frequently it grows on fence posts, pickets, and a variety of other structural timbers. Both species were formerly frequently referred to the genus *Trametes,* but it seems best to restrict that genus to annual forms only.

Fig. 7. Hyphae of *F. ohiensis.*

Besides the host distinction, other characters may be used to distinguish between the two species. In typical specimens of *F. scutellatus* the pileus is entirely black and attached dorsally to the under side of branches. *F. ohiensis* is rarely found so attached, and the whole plant is at first white, the upper or basal part of the pileus becoming blackish with age, as in many species of *Fomes,* but the margin remaining white, even in perennial forms. *F. scutellatus* is rarely ungulate in form, while old specimens of *F. ohiensis* become steep in front, much as in *F. fomentarius.*

The spores of *F. scutellatus* have never been recorded and Lloyd has recently stated[1] that he has failed to find them even in freshly collected material. Murrill records them as

[1] Syn. Fomes, p. 218. 1915.

"*smooth, hyaline,*" but that conclusion is reached only from inference. I find them to be cylindric, hyaline, smooth, 8–9 × 2.5–3.5 μ. They thus differ from those of *F. ohiensis,* which are ovoid with a truncate base, hyaline, smooth, 10–12 × 6–7 μ. It is apparent then that the spores of *F. ohiensis* are similar in shape to those found in all species recently segregated into the genus *Ganoderma,* while those of *F. scutellatus* point to an alliance of that species with the genus *Trametes,* they being typical trametoid spores.

Fig. 8. Hyphae of *F. scutellatus.*

It is only in rare cases that the branching of the hyphae of the context can be used as a distinguishing character. The hyphae of *F. scutellatus* are much branched, while those of *F. ohiensis* are practically simple. These differences are shown in figs. 7 and 8.

It is thus apparent that these closely related species are separated by rather wide differences, and their determination need no longer be considered difficult.

The following descriptions are appended:

1. **Fomes scutellatus** Schw. ex Cooke.

Pileus *convex,* sometimes attached by the vertex and circular in outline, 0.5–1.5 × 0.5–2 × 0.1–0.5 cm., *entirely dark brown or black,* at least when mature, slightly sulcate; context corky, about 2 mm. thick; tubes 1–2 mm. long, the mouths white or pallid, averaging 4–5 per mm., thick-walled; spores *cylindric, 8–9 × 2.5–3.5 μ*; cystidia none; hyphae hyaline to light brown, *much branched,* 2–4 μ; basidia 6–9 μ broad.

Usually growing on alder and witch-hazel.

Specimens examined: Ell. & Ev. N. Am. Fung. 1597 (Pennsylvania); Fung. Col. 1010 (Vermont).—Mo. Bot. Gard. Herb. 4469 (New Jersey).—Burt Herb. (collections from New York and Vermont).—Overholts Herb. 337 (Ohio), 2394 (Florida). Also reported from Maine, Delaware, and Alabama.

2. Fomes ohiensis Berk. ex Murrill.

Pileus *convex to ungulate,* sometimes attached by the vertex and circular in outline, 0.5–2.5 × 0.5–3 × 0.2–1 cm., *pure white, then black at the base, the margin remaining white,* often zonate or sulcate; context corky or woody, 1–3 mm. thick; tubes 1–4 mm. long, the mouths white, averaging 3–5 per mm., thick-walled; spores[1] *ovoid with a truncate base, 10–12 × 6–7 μ*; cystidia none; hyphae hyaline, *unbranched,* 3–4 μ; basidia 8–11 μ broad.

On dead wood and on structural timbers.

Specimens examined: Ell. N. Am. Fung. 923 (as *Trametes*) (Ohio).—Burt Herb. (collection from South America, ex Herb. Romell).—Overholts Herb. 38, 39, 131, and others (Ohio), 479 (Missouri), 503 (Illinois). Also reported from Kansas, Michigan, and New York.

Trametes Pini Thor. ex Fries, T. Abietis Karst., and T. piceinus Peck.

Trametes Pini dates from the year 1803, when it was described by Thore,[2] and again in the following year, 1804, by Broteri.[3] The typical form of the perennial plant is rather large, has a more or less ungulate pileus, and in age becomes blackish and rimose. At times, however, the first year's growth is thin and applanate and thus differs markedly in form from the typical plant. This condition was observed by Peck and the name *Polyporus* (later changed to *Trametes*) *piceinus* was proposed by him for the form that he collected on *Picea* about 1889.[4] Karsten had already[5] described the same plant in Europe, in 1882, as *Fomes Abietis,* and the two names have been used interchangeably in this country for several years. In 1889 Karsten[6] referred to his species as

[1] According to Murrill (N. Am. Flora 9: p. 96. 1908) the spores of the size and form given here are conidial, but they represent the only type of spore I have been able to find in the hymenium of this species.

[2] Chlor. Land. p. 487. 1803.

[3] Fl. Lusit 2: p. 468. 1804.

[4] Rept. N. Y. State Mus. 42: p. 121. 1889.

[5] Bidrag Finl. Nat. Folk. 37: p. 242. 1882.

[6] Finl. Basidav, p. 336. 1889.

Trametes Pini var. *Abietis,* and that name has also appeared in the American literature. The writer has not seen Karsten's types and his opinion as to the synonymy of the species of Peck and Karsten is based entirely on the use of the names in this country and on the fact that *T. Pini* var. *Abietis,* as distributed by Romell,[1] is certainly to be referred to Peck's species. In the case of *Polyporus piceinus* and *Trametes Pini,* however, the evidence is not so clear, and there are yet mycologists who distinguish between the two species.

Peck has stated[2] that the pileus of *T. piceinus* is persistently tomentose, while that of *T. Pini* is not tomentose, and on this ground and also in view of the fact that the former is thin and applanate while the latter is thick and ungulate, the two have been kept apart to some extent, though Murrill, in 1908, declared them to be not specifically distinct. During the summer of 1913 and again in 1914 the writer had the privilege of collecting in the almost unexplored (mycologically) region of the Rocky Mountains in central Colorado. Here the forests are principally composed of the lodge-pole pine (*Pinus Murrayana*) and the Engelmann spruce (*Picea Engelmannii*), the former genus being the typical host of *T. Pini* and the latter the same for *T. piceinus.* No extensive field observations had been previously reported as to the intermingling of these supposed species of fungi, and the opportunity was taken to procure some notes on the subject. In that region the species is more abundant on the spruce than on the pine, probably because the best spruce forests follow the courses of the streams, while the pine often represents the only tree growth on the mountain sides and in the higher parts of the mountain parks where the soil often contains a higher percentage of sand. Such forests are not dense and quickly become dry, unless kept moist by daily rainfalls. Hence the statement that *T. Pini* is more often found on spruce in that locality is not surprising. In one instance in an area of no more than four square feet on a spruce snag the writer counted 18 sporophores, and of these about half were the *T. Pini* form and

[1] Fung. Scand. 7.

[2] Rept. N. Y. State Mus. 54: p. 170. 1901.

the rest were good specimens of the thin form known as *T. piceinus*. There is no doubt in the writer's mind that all these sporophores came from a common mycelium. In 1914 a similar find was made, the substratum being an old spruce log. Portions of these two collections are preserved in the writer's herbarium. Attempts were later made to separate the specimens in these collections by means of microscopic characters, but it was found to be impossible. In view of these observations it is seen that the recently expressed opinion of Meinecke[1] that the variation in shape is due to the host, is not true for the fungus, as it sometimes occurs in Colorado.

In some localities it may be more convenient to consider the thin form as a variety of *T. Pini,* for it must be admitted that the two forms do not always grow in such close association as described above. Yet the evidence is clear that they cannot be regarded as distinct species.

The writer believes that it will add to the clearness of the general situation in the *Polyporaceae* to include in the genus *Fomes* all perennial plants of whatever structure. This not only simplifies the definition of the genus *Fomes,* but also gives a clearer idea of the genus *Trametes.* As it has been commonly understood, the genus *Trametes* is a very poorly defined one, and any attempt to make its limits clearer is a step in the right direction. The transfer of this species to *Fomes* has already been made by Lloyd[2]. The species is here described under that name.

1. Fomes Pini Thor. ex Lloyd.

Sporophores very variable, the variations grouping themselves as follows:

Typical form: Sporophore *perennial, often ungulate, 6–15* × *4–20* × *1–15* cm., at first tawny and with elevated zones of appressed tomentum, becoming blackish and glabrous, the surface cracking or becoming rough and irregular; context not more than 5 mm. thick, tawny or ochraceous tawny, woody; tubes 2–6 mm. long each season, the mouths ochraceous to

[1] Forest tree diseases common in California and Nevada, p. 43. 1914.

[2] Syn. Fomes, p. 275. 1915.

brown; spores globose or subglobose, hyaline, 4–5 μ broad; setae abundant, sharp-pointed, brown, extending 20–30 μ beyond the basidia; hyphae 3–5 μ.

Var. *Abietis* Karsten: Sporophores *usually annual, rather thin and applanate, 1–5 × 1–7 × 0.3–1* cm., tawny or russet-tawny toward the margin, the immediate margin sometimes brighter-colored, zonate with elevated ridges of tomentum, grayish black or brownish black toward the base; context colored as in the typical form, 1–3 mm. thick; tubes *usually in a single layer;* spores, setae, and hyphae, as in the typical form.

On wood of coniferous trees, both living and dead.

Illustrations: Boudier, Ic. Myc. *pl. 161.*—Delacroix, Atlas Path. Veget. *pl. 19. f. 10–12.*—Meinecke, For. Tree Dis. Calif. and Nev. *pl. 4–5.*—Rostk. in Sturm's Deutsch. Fl. **3**: fasc. 17. *pl. 50.*

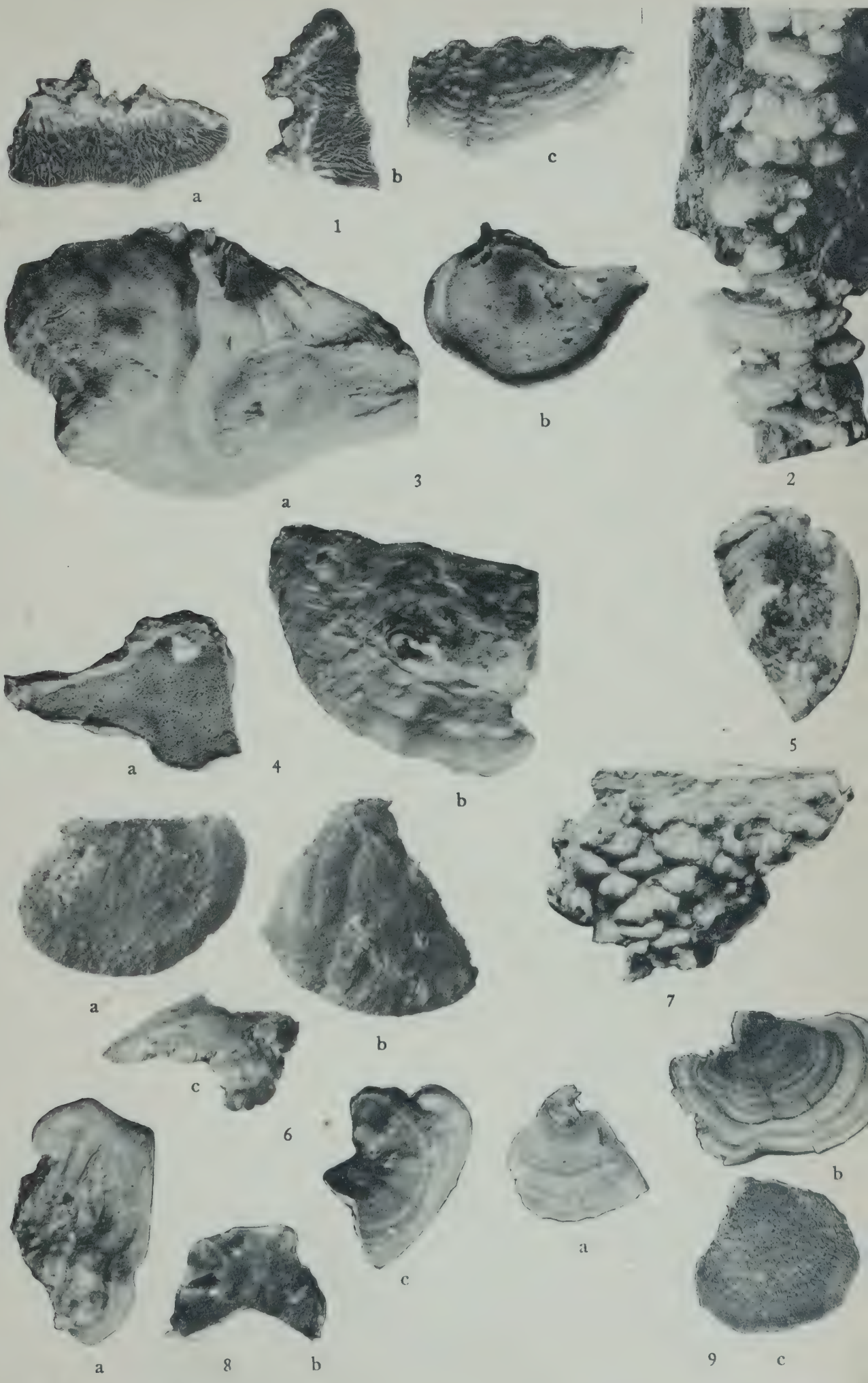
Specimens examined: Ell. N. Am. Fung. 602 (New Jersey).—Ell. & Ev. N. Am. Fung. 2507 (as *T. Abietis*) (Canada).—Linh. Fung. Hung. 348.—Rabenh. Crypt. Samm. Schule & Haus 8; Herb. Myc. 118.—Romell, Fung. Scand. 7 (as *T. Pini* var. *Abietis.*—Seym. & Earle, Econ. Fung. **11**: 549.—Mo. Bot. Gard. Herb. 42958 (Washington), 4609 (Newfoundland), 42970 (Maine), 42954 (Michigan), 42956 (Vermont), 4618 (Colorado), 43810 (Missouri), and others.—Overholts Herb. 154 (Ohio), 630, 2033, 642, and 2391 (Colorado), 2458 (Montana), and others.

Graduate Laboratory, Missouri Botanical Garden.

Explanation of Plate

PLATE 23

Fig. 1. Specimens of *P. abietinus* with lamellate hymenium.
Fig. 2. Surface view of typical sporophores of *P. abietinus*.
Fig. 3. Typical sporophores of *P. fumosus*.
Fig. 4. *P. Burtii*. Photograph of type specimens.
Fig. 5. Upper surface of *P. albellus*.
Fig. 6. *P. fumidiceps*, showing upper surface and section through a sporophore.
Fig. 7. *P. crispus*, showing the densely imbricate mode of growth and the pubescent pileus.
Fig. 8. *P. adustus*. View of surface of pileus and hymenium.
Fig. 9. Typical sporophores of *P. pargamenus*.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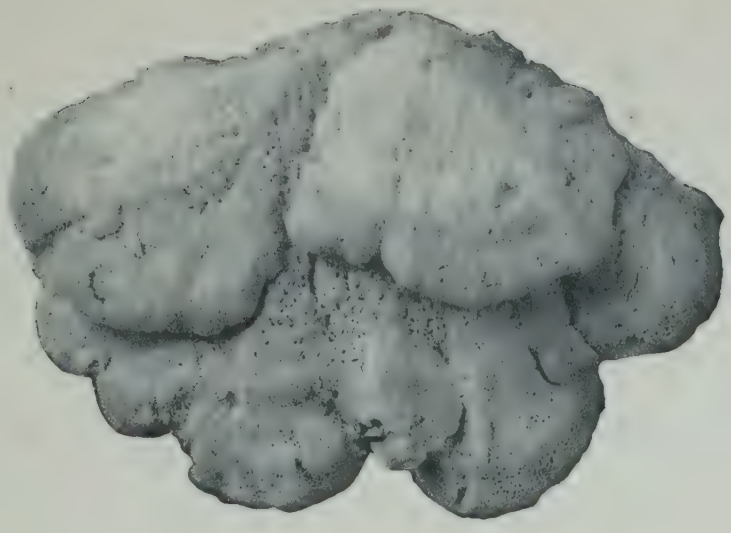
Explanation of Plate

PLATE 24

Fig. 10. View of hymenium of *P. spumeus.*
Fig. 11. The pores of *P. spumeus* somewhat enlarged.
Fig. 12. Section through a sporophore of *P. galactinus.* Note the prominent zonation of the context.
Fig. 13. Upper surface of *P. chioneus.*
Fig. 14. Comparison of the size of the tubes in (a) *P. spumeus* and (b) *P. delectans.*
Fig. 15. Upper surface of *P. galactinus.* Note the prominent pubescence.
Fig. 16. Sections showing the relative thickness of the pilei in (a) *P. albellus* and (b) *P. chioneus.*
Fig. 17. Hymenium of *P. galactinus.*



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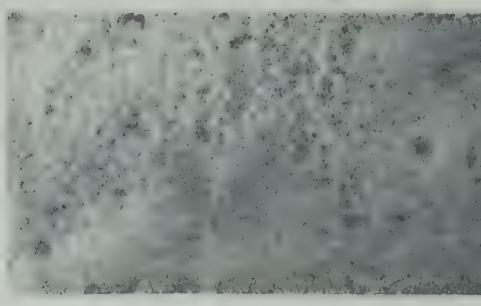

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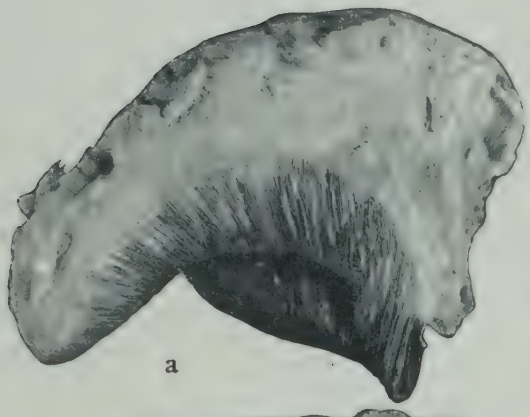

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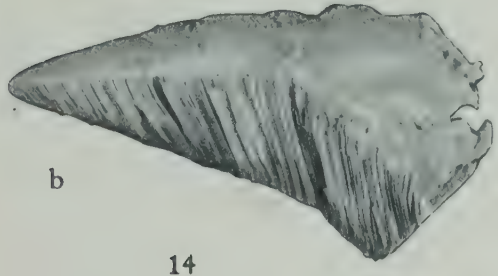

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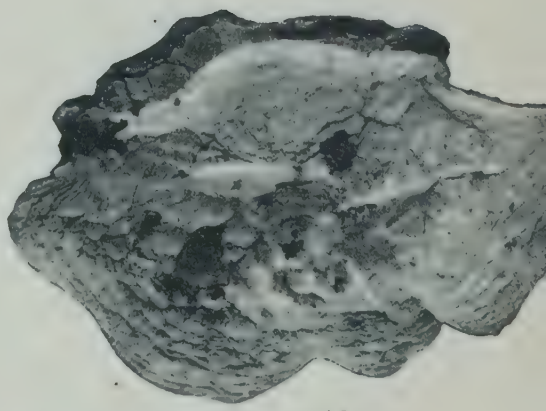

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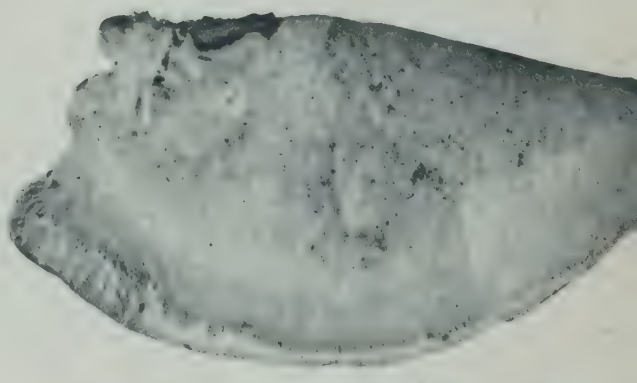

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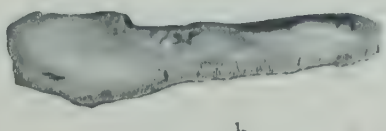

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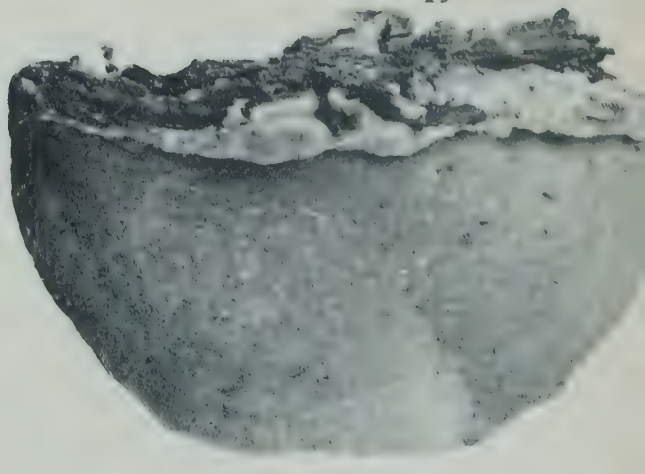

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Explanation of Plate

PLATE 25

Fig. 18. Sporophore of *F. igniarius* growing on beech trunk.

Fig. 19. View of upper surface and section through a sporophore of *F. fraxinophilus.*

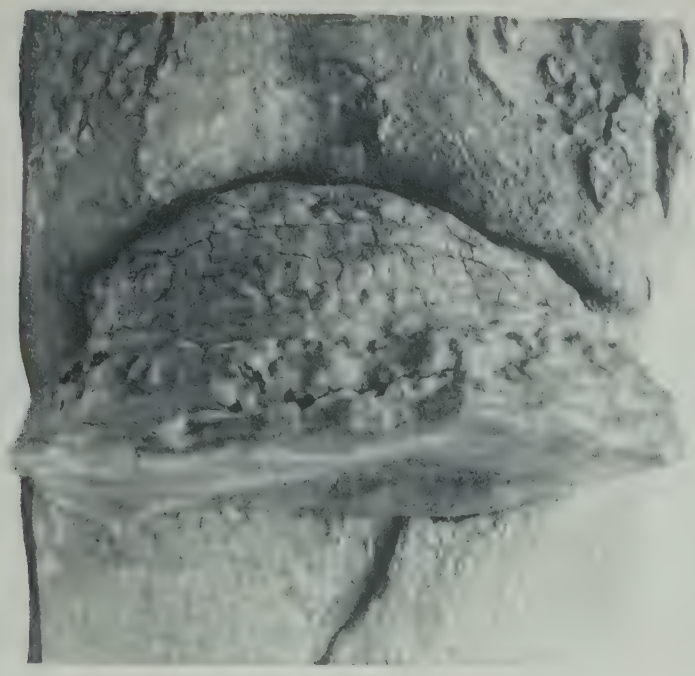
Fig. 20. Section through a typical sporophore of *F. igniarius* var. *nigricans.* Note the strongly white incrusted layers of tubes and context.

Fig. 21. Surface view of the same specimen of *F. igniarius* var. *nigricans.*

Fig. 22. Sporophores of *F. ohiensis.*

Fig. 23. *F. Ellisianus,* showing rugose upper surface and section of hymenium with long tubes.

Fig. 24. Sporophores of *F. scutellatus* on limbs of alder.



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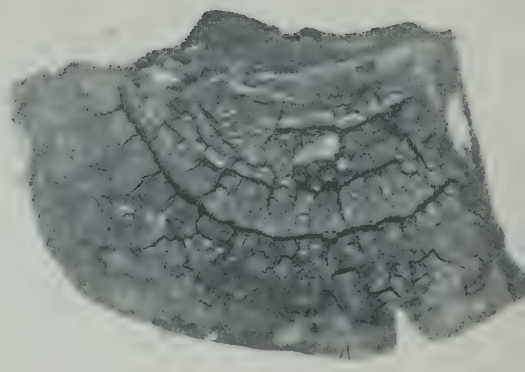

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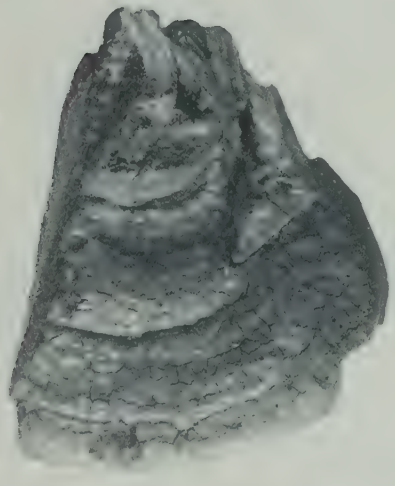

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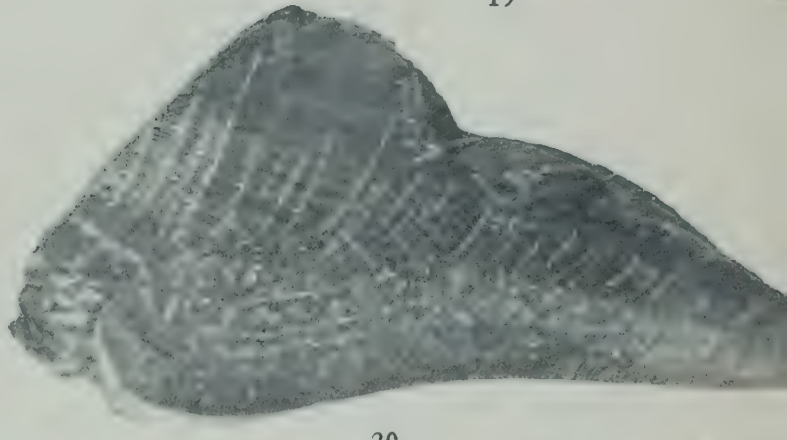

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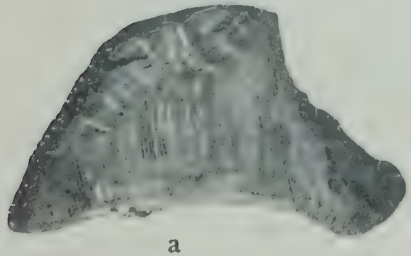

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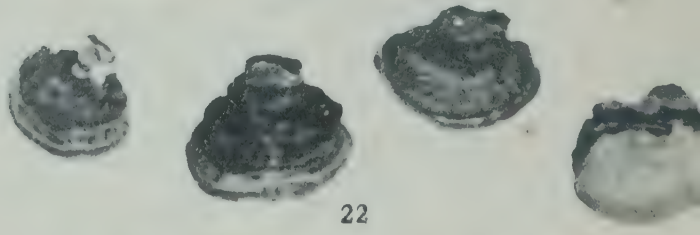

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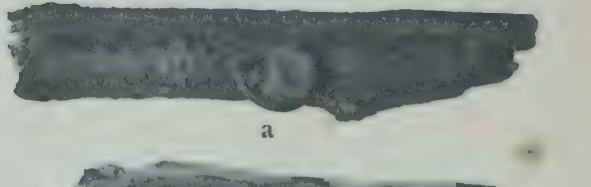

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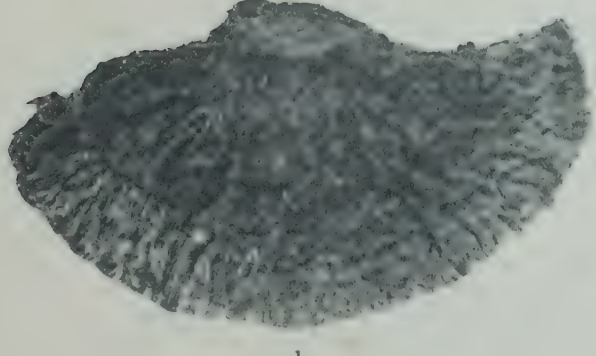

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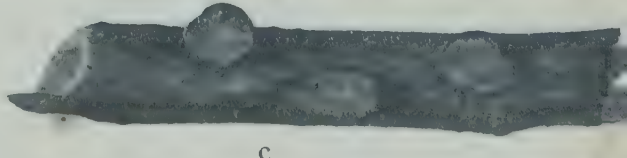

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24

THE THELEPHORACEAE OF NORTH AMERICA V[1]

Tremellodendron, Eichleriella, and Sebacina

EDWARD ANGUS BURT

Mycologist and Librarian to the Missouri Botanical Garden
Associate Professor in the Henry Shaw School of Botany of Washington University

The group of fungi comprising the present part probably attains its greatest development both in form and numbers in the western continent where it culminates in the erect *Tremellodendron,* apparently confined to North America. This continent has five of the seven species of *Eichleriella;* it has twenty-six species of *Sebacina* against fifteen for the Old World.

The better-known species of these genera were originally described in *Thelephora, Stereum,* and *Corticium,* with which they conform so closely in general habit of growth and consistency of the fructification that it is impossible to separate them from the latter except by microscopic examination of preparations which show the mature basidia to be longitudinally cruciately septate. Collectors invariably roughly grade their findings of *Sebacina* as *Corticium.* The recognition of longitudinally septate basidia is not always easy with the aid of the microscope; for example, the fungus originally described as *Stereum Leveillianum* B. & C. has been studied critically at several times by experts without their observing the true structure of the basidia.

I regret that the present account of our species and their range in North America does not include all the material at hand. The Missouri Botanical Garden herbarium contains several hundred undetermined specimens of possible Corticiums which have been received during the last two years.

Note.—Explanation in regard to the citation of specimens studied is given in Part I, Ann. Mo. Bot. Gard. 1 : 202. 1914, footnote. The technical color terms used in this work are those of Ridgway, Color Standards and Nomenclature. Washington, D. C., 1912.

[1] Issued December 20, 1915

I have looked through these collections very carefully to sort out, without examination now of everything by microscopic methods, just those specimens which ought to be studied at once for citation in this part, but some of the specimens most desirable for citation have undoubtedly been deferred for the present as probable Corticiums.

As it is really a nice microscopical task to recognize longitudinally septate basidia when they are not at their best, some notes, based on my experience, may be helpful. Species of *Tremellodendron* are the most easily recognized, for a little of the moistened and softened hymenium may be picked out with a scalpel, placed in a drop of water, stained with aqueous solution of eosin, 7 per cent potassium hydrate solution added, and then crushed down by pressure on the cover glass. In the detection of species of *Eichleriella* and *Sebacina,* thin vertical sections of the fructification are necessary. After the sections have been made turgid and clear by potassium hydrate solution, the latter should be drained off and the sections stained by merely a sufficient amount of solution of Gruebler's eosin soluble in alcohol, and mounted in water for temporary examination. It may be necessary to spread apart the tissues of the preparation somewhat by pressure upon the cover glass. If the preparation is to be preserved permanently in glycerin, a drop of dilute solution of sodium chloride should be run under the cover glass before the glycerin is added to insure a permanent stain by the Gruebler eosin.

Longitudinally cruciately septate basidia are simple and pyriform or subglobose when young, but so are the probasidia of *Septobasidium,* the possible storage organs of *Corticium polygonium,* and the basidia of some species of *Corticium.* The basidia of the latter are likely to form a layer at the surface of the fructification and are certainly simple if any can be detected bearing sterigmata and perhaps spores while still non-septate. In a fructification having longitudinally septate basidia, the hymenial surface is usually composed of paraphyses and of long, slender sterigmata arranged side by side; in this surface layer—but sometimes at a con-

siderable distance from the surface, as in *Thelephora Helvelloides* Schw.—is situated the layer of basidia. Only very rarely do the basidia of *Sebacina* or *Eichleriella* constitute the surface of the fructification.

If a fructification contains a palisade layer of deeply staining, pyriform bodies among or underneath the paraphyses and with no simple basidia in the surface layer, more or less prolonged examination of the pyriform bodies is likely to show longitudinal septa in some of them.

The three genera which comprise the present part of this monograph, are treated here by the writer, because their general habit and consistency conform so closely with *Thelephoraceae* having simple basidia, that they may be regarded as a connecting group, although belonging with the *Tremellaceae* by the structure of their basidia. Such of the species as were described in the past were described as *Thelephoraceae* or by authors with special knowledge of the *Thelephoraceae;* the taxonomic recognition of fungi of these genera seems likely to continue to fall in the future to students of the *Thelephoraceae,* for other mycologists will hardly care to glean for material of so few species among the many *Thelephoraceae* of similar aspect.

TREMELLODENDRON

Tremellodendron Atkinson, Jour. Myc. **8**: 106. 1902; Saccardo, Syll. Fung. **17**: 208. 1905.

The type species is *Merisma candidum* Schw.

Fructifications coriaceous, erect, pileate, branched or rarely simple; hymenium amphigenous or inferior; basidia longitudinally cruciately septate; spores white, even.

The species of *Tremellodendron* are indigenous to North America; none have been reported for other regions, so far as I am aware. The fructifications spring up on the ground in deep woods during wet weather in summer and early autumn, and have the general habit of *Thelephora vialis,* of branched Clavarias, or, very rarely, of simple clubs. In active vegetative condition the fructifications may be distinguished from species of *Clavaria* of similar habit by coriaceous and

tough consistency and by lack of brittleness. The longitudinally septate basidia afford a decisive character in all doubtful cases.

The specific distinctions between the more common species of this genus are based largely upon the form of mature and well-developed fructifications; very young, deformed, or fragmentary specimens can not be referred very confidently to their species.

Key to the Species

Fructifications branched when well developed. Simple forms may be present when very young or in the same colony with normal branched forms 1
Fructifications simple 4
1. Fructifications normally cespitose, more or less grown together..... 2
1. Fructifications solitary or scattered..... 3
2. With pileate divisions flattened, grown together at many points of contact, forming rosette-like masses 2–15 cm. in diameter..*1. T. pallidum*
2. With the stems grown together into a main stem 2–10 mm. thick; pileate divisions cylindric, spreading, grown together at only few points of contact; the smaller divisions about 1½ mm. thick..... .....*2. T. candidum*
2. Sometimes with both stems and pileate divisions grown together into compact bundles, usually merely closely cespitose and with the branches intricately intertangled; much slenderer than preceding species and with the habit of *Pterula*.....*5. T. merismatoides*
3. Stem about 1½ mm. thick, palmately few-branched; branches once or twice similarly branched, cylindric or subcylindric, often channelled on the upper side; basidia 15×9 μ; spores 9–15×4½–6 μ, pointed at the base only .....*3. T. Cladonia*
3. Stem about ½–1 mm. thick, sometimes with occasional, scattered, divergent branches from its side, dilated at the upper end, divided into a few, short, finger-shaped branches; basidia 20–24×12–14 μ; spores 14–16×6–7 μ, pointed at both ends, Known from Jamaica only.....*4. T. tenue*
4. Fructification dark orange, probably with medullary tissue pale as in all the preceding species; basidia subglobose, 10–12 μ in diameter .....*6. T. aurantium*
4. Fructification black with the exception of the hymenium; hymenium olive-ocher, amphigenous on the lower third of the fructification; basidia 11×7 μ. Known from Porto Rico only.....*7. T. simplex*

1. **Tremellodendron pallidum** (Schw.) Burt, n. comb.

Plate 26, fig. 6.

Thelephora (*Merisma*) *pallida* Schw. Am. Phil. Soc. Trans. N. S. **4**: 166. 1834.—*T. Schweinitzii* Peck, N. Y. State Mus. Rept. **29**: 67. 1878; Saccardo, Syll. Fung. **6**: 534. 1888.—*Tremellodendron Schweinitzii* (Peck) Atk. Jour. Myc. **8**: 106. 1902.

Illustrations: Hard, Mushrooms *f. 381.*—Moffatt, Chicago Acad. Sci. Bul. 7: *pl. 22. f. 1.* 1909.

Type: in Herb. Schweinitz and a portion in Curtis Herb.

Fructification cespitose, erect, white or pallid, drying warm buff, stipitate by one to several or many stems which may be distinct below or arise from a common, swollen, basal mass; above, the stems branch into flattened, more or less furrowed, pileate divisions which grow together at surfaces of contact to form a somewhat cup-shaped or rosette-like mass; divisions in center of mass somewhat subulate at the apex, those at margin dilated and sometimes fimbriate, splitting when dry into sharp fibers or spicules; hymenium inferior, warm buff, best developed towards the base of the pileate divisions; basidia pyriform, longitudinally cruciately septate, 12–15 × 9 μ; spores from a spore collection, white, simple, 10–12 × 4½–5½ μ, and 9–12 × 4½ μ from an herbarium specimen.

Fructifications 2–10 cm. high, 2–15 cm. broad.

On the ground in deep woods. Canada to South Carolina and westward to Missouri. June to October. Common.

Full-grown and well-developed specimens are rosette-like and resemble *Thelephora vialis* when viewed from above but may have the pileate mass supported by many stems; small specimens with only a single stem do occur. The large specimens are apparently due to the concrescence of many small fructifications. In the large specimens the pileate divisions on the outside of the mass become broader and more flattened than those in the interior. The flattened form of the divisions of the pileus and their growing together at numerous points of contact are characters separating *Tremellodendron pallidum* from *T. candidum*. The small specimens, distributed as *T. pallidum* in published exsiccati, are often so immature and fragmentary that they cannot be distinguished from *T. candidum*.

Forms of *T. pallidum* which have the tips of pileate divisions split into sharp fibers or spicules are the *Thelephora cristata* and *T. serrata* of Schweinitz, 'Syn. N. Am. Fungi,' Nos. 621 and 623.

Specimens examined:

Exsiccati: Ravenel, Fungi Car. II, 29; Ellis, N. Am. Fungi, 510; Ell. & Ev., Fungi Col., 1208; Shear, N. Y. Fungi, 50.

Canada, Ontario: London, *J. Dearness,* and also in Ell. & Ev., Fungi Col., 1208; Belleville, *J. Macoun, 174, 230* (both in Can. Geol. Surv. Herb.).

Maine: N. Parsonfield, *R. G. Leavitt.*

Vermont: near Burlington, *L. R. Jones,* two collections; Middlebury, *E. A. Burt,* two collections.

Massachusetts: *Sprague, 773* (in Curtis Herb. under the name *Thelephora vialis*); Brookline, *S. Davis.*

Connecticut: East Hartford, *C. C. Hanmer;* and also No. 1567 (in Hanmer Herb.).

New York: Alcove, *C. L. Shear,* N. Y. Fungi, 50; Floodwood, *E. A. Burt;* Taughannock, *H. H. Whetzel,* Cornell Univ. Herb., 13600; Buffalo, *G. W. Clinton* (in U. S. Dept. Agr. Herb.); Tarrytown, *H. von Schrenk* (in Mo. Bot. Gard. Herb., 42800).

New Jersey: Laning (in Mo. Bot. Gard. Herb., 701330, 701331, 701333); Newfield, *J. B. Ellis* (in Mo. Bot. Gard. Herb., 5162), and also N. Am. Fungi, 510.

Pennsylvania: Bethlehem, *Schweinitz,* type (in Herb. Schweinitz, and a portion in Curtis Herb. and also the Nos. 621 and 623 of Schweinitz, 'Syn. N. Am. Fungi,' under the names *Thelephora cristata* and *T. serrata,* respectively); Trexlertown, *W. Herbst* (in Lloyd Herb.); Kittanning, *D. R. Sumstine.*

Delaware: Newark, *H. S. Jackson, B10.*

District of Columbia: Washington, *O. F. Cook, 2,* comm. by P. L. Ricker.

Virginia: Great Falls, *C. L. Shear, 1044.*

North Carolina: Blowing Rock, *G. F. Atkinson,* Cornell Univ. Herb., 10666, 10667, 10669, 10664 (of which the first two numbers and part of the third are in Cornell Univ. Herb. and part of the third and the last in Mo. Bot. Gard. Herb.).

South Carolina: Ravenel, Fungi Car. II, 29.

Ohio: *C. G. Lloyd, 2346* (in Lloyd Herb.); Loveland, *D. L. James* (in U. S. Dept. Agr. Herb.).

West Virginia: Eglon, *C. G. Lloyd, 02601.*

Kentucky: *S. M. Price* (in Mo. Bot. Gard. Herb., 5141, 5144, 701332, 712372); Mammoth Cave, *C. G. Lloyd, 1071.*

Illinois: *H. C. Beardslee* (in Lloyd Herb., 2175); Newton's Ferry, *E. T. & S. A. Harper, 441;* Riverside, *E. T. & S. A. Harper, 696.*

Wisconsin: Blanchardville, Univ. of Wis. Herb., 52; Madison, *E. T. & S. A. Harper, 881; C. J. Humphrey, 948* (in Mo. Bot. Gard. Herb., 44783).

Iowa: *T. J. Fitzpatrick* (in Lloyd Herb.).

Missouri: St. Louis, *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 701335, 701370, 701371); Cliff Cave, *J. B. S. Norton* (in Mo. Bot. Gard. Herb., 5126); Columbia, *B. M. Duggar, 140;* Creve Coeur, *Miss E. M. Briggs* (in Mo. Bot. Gard. Herb., 44756).

2. T. candidum Schw. ex Atkinson, Jour. Myc. **8**: 106. 1902. Plate 26, fig. 3.

Merisma candidum Schweinitz, Naturforsch. Ges. Leipzig Schrift. **1**: 110. 1822.—*Thelephora candida* Fries, Elenchus Fung. 168. 1828; Schweinitz, Am. Phil. Soc. Trans. N. S. **4**: 166. 1834.

Type: in Herb. Schweinitz, Acad. Nat. Sci. Phila.

Fructifications cespitose, erect, coriaceous-soft, white, drying warm buff, stipitate; stem thick, palmately branched, with branches spreading, branching, cylindric or subcylindric; hymenium inferior on the main branches, often amphigenous on secondary branches; basidia longitudinally septate, 10–12 × 7½–9 μ; spores colorless, simple, even, 7½–10 × 4½–5½ μ.

Fructifications 2½–5 cm. high, 2–5 cm. broad; stem 2–10 mm. thick; smaller pileate branches about 1½ mm. thick.

On ground in open woods. Vermont to North Carolina and westward to Missouri. July to September. Infrequent.

The type of *T. candidum* has the dimensions given above for recent collections. In the original description Schweinitz noted that fructifications may attain a breadth of 15 cm.; at that time he had not given specific recognition to the large and common *T. pallidum* and it may be that the large specimens to which he referred were of the latter species. *T. candidum* is closely related to *T. pallidum* but contrasts with the latter in having consolidation between adjacent fructifications

confined to the main stems from the base upward to about the region of branching; from here the branches spread so that they grow together only rarely; furthermore, the branches are distinctly cylindric or subcylindric. The spores average a little shorter than those of related species.

Specimens examined:

Vermont: Lake Dunmore, *E. A. Burt;* Newfane, *C. D. Howe.*

Massachusetts: Woods Hole, *G. T. Moore.*

New York: Alcove, *C. L. Shear, 1218;* Fishers Island, *C. C. Hanmer, 192, 193, 194* (all in Hanmer Herb.).

North Carolina: *Schweinitz,* type (in Herb. Schweinitz); Blowing Rock, *G. F. Atkinson,* Cornell Univ. Herb., 10662, 10668 (in Mo. Bot. Gard. Herb., 44775, 44776) and (in Cornell Univ. Herb., 10663).

Ohio: Granville, *H. L. Jones.*

Missouri: near St. Louis, *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 701336).

3. T. Cladonia (Schw.) Burt, n. comb. Plate 26, figs. 1, 2.

Merisma Cladonia Schweinitz, Naturforsch. Ges. Leipzig Schrift. **1**:110. 1822.—*Thelephora Cladonia* Fries, Elenchus Fung. 168. 1828; Epicr. 537. 1836–1838; Schweinitz, Am. Phil. Soc. Trans. N. S. **4**:166. 1834; Saccardo, Syll. Fung. **6**:535. 1888.—*Thelephora gracilis* Peck, Torr. Bot. Club Bul. **25**:371. 1898.

Type: in Herb. Schweinitz.

Fructifications solitary or gregarious, erect, coriaceous-soft, pallid, drying warm buff, sometimes with the older portions pale olive-gray, stipitate; stem cylindric, palmately branched into a few—often three—cylindric branches, each or some of which occasionally branch again in similar manner; branches arranged in a plane from flattened end of stem or branch or in a circle about the cylindric end of the stem which is then sometimes perforate and the branches often channelled; hymenium amphigenous, or inferior when the branch is channelled; basidia longitudinally septate, pyriform, 15×9 μ; spores colorless, simple, even, curved, 9–15 $\times$ $4\frac{1}{2}$–6 μ.

Fructifications 2½–5 cm. high, 7 mm.–2 cm. broad; stem about 1½ mm. thick.

On ground in woods. Canada to Mississippi and westward to Missouri. August and September.

The fructification of this species is smaller than that of *T. candidum* and has but few branches, which are often arranged in a circle about the end of the stem so as to appear somewhat proliferous on the margin of an imperfect cup as in some species of the lichen, *Cladonia*—hence the specific name—or with the branches standing up side by side from the compressed apex of the main stem. Both forms of branching have been found so associated in the same collection as to preclude the possibility of regarding this difference as a basis for two species. The branches are so frequently in threes that "trifaria" was contemplated as a name for the species by one author.

Specimens examined:

Canada: *J. Macoun, 78.*

Vermont: Smugglers Notch, *L. R. Jones;* Middlebury, *E. A. Burt;* Brattleboro, *C. C. Frost* (in Univ. Vermont Herb.).

Massachusetts: *Sprague, 871* (in Curtis Herb., 5762).

New York: Hague, *C. H. Peck, 7;* Ithaca, *G. F. Atkinson,* Cornell Univ. Herb., 7708.

Pennsylvania: Trexlertown, *W. Herbst* (in Lloyd Herb.).

District of Columbia: Takoma Park, *P. L. Ricker, 822* (in Ricker Herb.).

North Carolina: *Schweinitz,* type (Herb. Schweinitz and a portion in Curtis Herb.); Blowing Rock, *G. F. Atkinson* (in Cornell Univ. Herb., 10665, 10008. A part of the latter number is in Mo. Bot. Gard. Herb., 44774).

Georgia: Tallulah Falls, *A. B. Seymour,* Farlow Herb., O, P, Q, R, U, W (in Mo. Bot. Gard. Herb., 44619, 44623–44625, 44628, 44630).

Alabama: *F. S. Earle, 13,* type of *Thelephora gracilis* (in Coll. N. Y. State).

Mississippi: Biloxi, *Mrs. F. S. Earle, 32A.*

Ohio: Cincinnati, *A. P. Morgan* (in Lloyd Herb., 32); Loveland, *D. L. James.*

West Virginia: Eglon, *C. G. Lloyd, 02634.*
Missouri: Creve Coeur, *E. A. Burt* (in Mo. Bot. Gard. Herb., 44755).

4. T. tenue Burt, n. sp. Plate 26, fig. 7.

Type: in Burt Herb. and in N. Y. Bot. Gard. Herb.

Fructifications scattered, erect, very slender, coriaceous-soft, drying warm buff, stipitate; stem equal, flexuous, drying somewhat twisted and flattened, becoming fibrillose, sometimes giving off two or three scattered, divergent, small branches, dilated above and divided in a few palmately arranged, finger-shaped branches; hymenium inferior on the dilated portion and branches; basidia longitudinally septate, 20–24 × 12–14 μ; spores colorless, simple, even, curved, pointed at both ends, 14–16 × 6–7 μ.

Fructifications 2–3½ cm. high, 3 mm. broad; stem 1½–2½ cm. long, about ½–1 mm. thick.

On the ground in wet mountainous region, altitude 3000–5200 ft. Jamaica. December and January.

This species is characterized by its long and slender stem, few branches, and the largest basidia and spores of any species of the genus. The spores differ from those of the other species in being pointed at the apex.

Specimens examined:
Jamaica: Chester Vale, *W. A. & E. L. Murrill,* N. Y. Bot. Gard., Fungi of Jamaica, 400, type; Cinchona, *W. A. & E. L. Murrill,* N. Y. Bot. Gard., Fungi of Jamaica, 614.

5. T. merismatoides (Schw.) Burt, n. comb. Plate 26, fig. 4.

Clavaria merismatoides Schweinitz, Am. Phil. Soc. Trans. N. S. **4**: 182. 1834.—*Merisma Schweinitzii* Leveille, Ann. Sci. Nat. Bot. IV. **5**: 157. 1846.—*Lachnocladium merismatoides* (Schw.) Morgan, Cincinnati Soc. Nat. Hist. Jour. **10**: 193. 1888.—*Pterula merismatoides* (Schw.) Saccardo, Syll. Fung. **6**: 742. 1888.—*Thelephora merismatoides* Lloyd, Letter No. 26: 2. 1909. Nomen nudum.—*Tremellodendron merismatoides* Lloyd, Letter No. 40: 2. 1912. Nomen nudum.—*Thelephora pteruloides* Berk. & Curt., Hooker's Jour. Bot. **1**: 238. 1849; Grevillea **1**: 148. 1873.

Type: In Herb. Schweinitz, Acad. Nat. Sci. Phila.

Fructifications erect, cespitose or fasciculate, and sometimes with stems grown together, coriaceous, branched, pallid, drying with stems warm buff and branches tawny; branches few, rather straight, filiform, angular-terete; branchlets many, dilated and fimbriate at the apex, then splitting into spreading branchlets; hymenium glabrous, amphigenous; basidia longitudinally septate, pyriform, 12–15 × 8–9 μ; spores in preparations from herbarium specimens hyaline, even, simple, 8–10 × 4½–5 μ.

Cluster of fructifications 2–5 cm. high, 2–3 cm. broad. Individual from cluster has stem 5–10 mm. long, ½–1 mm. thick; branches about ¼–⅓ mm. thick.

On the ground in open woods. Massachusetts and New York to South Carolina and westward to Missouri. June to August.

This is a small species with the habit of a *Pterula* but with coriaceous structure and longitudinally septate basidia. The fructifications of a cluster may have their stems distant from one another by spaces equal to the diameter of the stems, but the branches interlock above; in other cases the fructifications are crowded closely together and united throughout their whole length. *T. merismatoides* may be distinguished from the preceding species by the smaller diameter of the stems and branches and from all the following species by its cespitose to fasciculate habit.

The collection from West Virginia, distributed as *Thelephora pteruloides* in Ell. & Ev., 'N. Am. Fungi,' 3415 and 'Fungi Col.,' 1117, has the hymenium composed of basidia standing side by side in a distinct palisade layer and the basidia not longitudinally septate in my opinion.

Specimens examined:

Massachusetts: near Boston, *Murray,* comm. by Sprague, 250 (in Curtis Herb. under the name *Thelephora pteruloides* B. & C.); Woods Hole, *G. T. Moore, 58.*

New York: Ithaca, *G. F. Atkinson, 37;* Fishers Island, *C. C. Hanmer, 1478* (in Hanmer Herb.).

New Jersey: Haddonfield, *T. J. Collins* comm. by C. G. Lloyd.

Pennsylvania: Bethlehem, *Schweinitz,* type (in Herb.

Schweinitz); York County, *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 44742); Kittanning, *D. G. Sumstine.*

South Carolina: *M. A. Curtis, 1745* (the type and cotype of *Thelephora pteruloides* in Kew Herb. and Curtis Herb. respectively).

Ohio: Cincinnati, *A. P. Morgan,* Lloyd Herb., 2589 (determined by Morgan as *Thelephora filamentosa*).

Wisconsin: Lake Geneva, *E. T. & S. A. Harper, 842.*

Missouri: Meramec Highlands, *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 44743).

6. T. aurantium Atkinson, Ann. Myc. **6**: 59. 1908.

Type: in Cornell Univ. Herb. but cannot be found at present.

"Plants simple, slender, 1–3 cm. long, 2–3 mm. stout, dark orange, tough. Basidia subglobose, 10–12 μ, longitudinally divided; sterigmata 4, long, slender, flexuous. Spores oboval-subelliptical, granular, then with an oil drop, 7–10 $\times$ 5–6 μ, white, hyaline.—C. U. herb., No. 10684, ground, woods, along small stream crossing Boone Road, Blowing Rock, Blue Ridge Mts., N. C. G. F. Atkinson, Aug. 19–Sept. 22, 1901."

—Original description.

T. aurantium differs from the preceding species of *Tremellodendron* by its simple fructifications. I have seen no specimens referable here. Professor Atkinson had intended to make a negative from his type so that I could include a figure of the species, but, upon going to the envelopes labelled *T. aurantium,* he found that they contained—by error of a helper—*T. merismatoides* instead. The specimens of *T. aurantium* have not been found.

7. T. simplex Burt, n. sp. Plate 26, fig. 5.

Type: in Mo. Bot. Gard. Herb. and in Farlow Herb.

Fructifications scattered, erect or suberect, drying hard, brittle, somewhat longitudinally wrinkled and sometimes compressed, black above, olive-ocher with the hymenium towards the base; hymenium amphigenous on the lower third of the fructification, olive-ocher, hyaline under the microscope, with surface consisting of colorless clavate paraphyses 5 μ thick,

and with basidia and spores at base of the paraphyses; basidia longitudinally septate; 11 × 7 μ; spores colorless, even, 7½–9 × 5–6 μ.

Fructifications about 2 cm. long, about 2 mm. thick.

In cane field. Porto Rico.

T. simplex is noteworthy by the column composed of longitudinally arranged, black hyphae, which extends the whole length of fructification and constitutes the whole, upper, sterile two-thirds of the fructification and is clothed by the ochraceous hymenium on the lower third. The specimens are broken off at the base, hence I cannot be sure that a stem was not originally present, but if present it would doubtless have been included in the packet. The general habit is that of a small *Geoglossum* or cylindric *Xylaria.*

Specimens examined:

Porto Rico: *J. R. Johnston,* comm. by W. G. Farlow, type (in Mo. Bot. Gard. Herb., 5119).

EICHLERIELLA

Eichleriella Bresadola, Ann. Myc. **1**:115. 1903.—*Hirneolina* as a section of *Sebacina* Patouillard, Essai Taxon. 24. 1900.—*Hirneolina* (Pat.) Saccardo, Syll. Fung. **17**: 208. 1905.

Fructifications coriaceous, waxy or membranaceous, subgelatinous, cup-shaped or plano-concave, rarely pendulous, hymenium typically superior, discoid, inferior in pendulous forms, even or somewhat rugulose; basidia globose-ovoid, cruciately divided, with 2–4 sterigmata; spores hyaline, cylindric, somewhat curved. It is a *Stereum* or *Cyphella* with tremellaceous hymenium.

The type species of the genus is *Eichleriella incarnata* Bres.

The original definition of *Eichleriella,* which is translated above, should be broadened to accurately describe our North American species, which are as coriaceous as *Stereum spadiceum.* All have the hymenium inferior. *Eichleriella gelatinosa* is our only species with subgelatinous hymenium.

But few species of this genus are known. Five species of *Eichleriella* have been recognized up to the present time in North America, three in Europe, and two in South America;

of our five, only one species, *Eichleriella Leveilliana,* ranges through the eastern United States; *E. spinulosa* occurs in both Europe and North America.

Key to the Species

Fructifications gray, small, ½–2 mm. long, ½–1 mm. broad, with habit of *Cyphella* ..... *1. E. Schrenkii*

Fructifications the color of raspberries and cream, and peltate at first, 1–5 cm. long, ½–1½ cm. broad..... *2. E. Leveilliana*

Fructifications ochraceous buff, 200–300 μ thick; hymenium even; known from Cuba and Brazil..... *3. E. alliciens*

Fructifications wood-brown, with whitish margin; hymenium dry, with tubercules like *Radulum*..... *4. E. spinulosa*

Fructification white at first, then clay-color, tomentose, soft and spongy, ½ cm. thick; hymenium gelatinous; known from Jamaica only..... *5. E. gelatinosa*

1. Eichleriella Schrenkii Burt, n. sp. Plate 27, fig. 8.

Type: in Mo. Bot. Gard. Herb. and in Farlow Herb.

Fructifications gregarious, coriaceous, sessile, pezizoid, oblong or rotund, margin free and strongly inrolled, pubescent, smoke-gray; hymenium concave, pale smoke-gray to pallid neutral gray; basidia longitudinally septate, pyriform, 22 × 11 μ; spores white in collection on slide, simple, curved, pointed at base, 12–19 × 6–7½ μ.

Fructifications ½–2 mm. long, ½–1 mm. broad, ½ mm. thick.

On bark of dead limbs of *Prosopis* (mesquite). San Antonio, Texas. February.

The general habit of this fungus resembles that of very small specimens of *Corticium Oakesii,* of large species of *Cenangium,* or of a sessile *Cyphella;* from all of which *Eichleriella Schrenkii* is easily separated by its longitudinally septate basidia which show clearly in sectional preparations. The fructifications are much smaller than those of any other species of this genus heretofore described.

Specimens examined:

Texas: San Antonio, *H. von Schrenk,* type (in Mo. Bot. Gard. Herb., 42579), and also (in Mo. Bot. Gard. Herb., 42580).

2. E. Leveilliana (Berk. & Curtis) Burt, n. comb. Plate 27, fig. 9.

Corticium Leveillianum Berk. & Curtis, Hooker's Jour.

Bot. 1:238. 1849.—*Stereum Leveillianum* Berk. & Curtis, Grevillea 1:163. 1873.

Type: type and cotype in Kew Herb. and in Curtis Herb., respectively.

Fructification coriaceous, soft, dry, rather thick, vinaceous fawn at first, whitening with age, resupinate-effused, with the margin free, sometimes narrowly reflexed, concolorous, minutely tomentose; hymenium composed of a surface layer about 30 μ thick of paraphyses $1\frac{1}{2}$–2 μ in diameter and of a layer of basidia under this; basidia longitudinally septate, 10–18 $\times$ 6–12 μ; spores in spore collection, white, simple, curved, pointed at base, 12–16 $\times$ 5–6 μ.

Fructifications often 5 mm. in diameter at first, finally up to 1–5 cm. long, $\frac{1}{2}$–$1\frac{1}{2}$ cm. broad, about $\frac{1}{2}$ mm. thick.

On dead limbs of several species. New York to Texas, Cuba, Jamaica, Central America, and Brazil. November to May.

This is a well-marked species upon which Berkeley made the following excellent graphic comment:

"At first forming little peltate orbicular spots, which, as they dilate, become closely attached to the matrix, with the exception of the margin, which is often free, soon confluent, soft, rather thick; of the colour of raspberries and cream. Hymenium often minutely pitted. Old specimens lose in great measure their ruddy hue, and are of a dead white."

I have seen no specimens having the hymenium minutely pitted.

Specimens examined.

Exsiccati: Ravenel, Fungi Car. II, 35.

New York: Hudson Falls, *S. H. Burnham* (in Mo. Bot. Gard. Herb., 44009, 44170, 44194); Buffalo, *G. W. Clinton.*

South Carolina: *M. A. Curtis, 1220, 92* (types and cotypes in Kew Herb. and Curtis Herb., respectively); *Ravenel,* Ravenel, Fungi Car. II, 35.

Georgia: Tallulah Falls, *A. B. Seymour,* Farlow Herb., C (in Mo. Bot. Gard. Herb., 44608).

Texas: Austin, *W. H. Long, 570,* Cornell Univ. Herb.; San

Antonio, *A. B. Langlois, bd;* same locality, *H. von Schrenk* (in Mo. Bot. Gard. Herb., 42576).

Cuba: San Diego de los Baños, *Earle & Murrill, 296, 356 in part,* N. Y. Bot. Gard. Herb.

Jamaica: Cinchona, *W. A. & E. L. Murrill,* N. Y. Bot. Gard., Fungi of Jamaica, 493.

Brazil: Blumenau, *A. Möller,* comm. by G. Bresadola; Matto Grosso Cuyaba, *G. Malme, 599,* comm. by L. Romell.

3. E. alliciens (Berk. & Cooke) Burt, n. comb.

Plate 27, fig. 10.

Stereum alliciens Berk. & Cooke, Linn. Soc. Bot. Jour. **15**: 389. 1876; Massee, Linn. Soc. Bot. Jour. **17**: 201. 1891.

Type: in Kew Herb.

Fructification coriaceous, resupinate, sometimes narrowly reflexed, separable, ochraceous buff, the margin slightly paler, the reflexed portion tomentose; structure in section, 200–300 μ thick, (1) with hyphae next to substratum ochraceous, loosely interwoven and protruded, 3 μ in diameter, similar to those on outer surface of reflexed portion, (2) with intermediate layer 100–180 μ thick, composed of longitudinally arranged hyphae 2 μ in diameter, (3) with hymenium composed of basidia 10 μ below the surface, imbedded in jelly through which rise a few filiform paraphyses or hyphae to the surface; hymenium even, ochraceous buff; basidia longitudinally cruciately septate, pyriform, 12–15 $\times$ 9–10 μ; spores colorless, simple, even, curved, 10–13 $\times$ $3\frac{1}{2}$–5 μ.

Fructifications of type described as several inches long, originally orbicular; Cuban specimen 1 cm. long, 1 cm. broad, reflexed side 1–2 mm. long, 1 cm. broad.

On dead wood in virgin forest. Cuba and Brazil. March.

The fructification resembles in habit and coloration that of a resupinate specimen of *Stereum hirsutum* with a very narrowly reflexed margin. The Cuban collection, of which but a single fructification was communicated to me, is much smaller than the Brazilian type and has the hyphae of the intermediate layer with gelatinously modified wall.

Specimens examined:

Brazil: San Antonio da boa vista, Rio Javary, *Traill, 1,* type (in Kew Herb.).

Cuba: San Diego de los Baños, Pinar del Rio Province, *Earle & Murrill, 405,* N. Y. Bot. Gard. Herb.

4. **E. spinulosa** (Berk. & Curtis) Burt, n. comb.

Plate 27, fig. 11.

Radulum spinulosum Berk. & Curtis, Grevillea **1**: 146. 1873.—*Radulum deglubens* Berk. & Broome, Ann. and Mag. Nat. Hist. IV. **15**: 32. 1875.—*Eichleriella deglubens* (Berk. & Br.) Lloyd, Letter No. 45: 7. 1893; Wakefield, Brit. Myc. Soc. Trans. **4**: 305. 1914.—*Stereum rufum* of English authors but not *S. rufum* Fries.—*Radulum Kmetii* Bresadola, I. R. Accad. degli Agiati Rovereto Atti III. **3**: 102. 1897.—*Eichleriella Kmetii* Bresadola, Soc. Myc. France **25**: 30. 1910.

Type: in Kew Herb.

Fructifications longitudinally and broadly effused, wood-brown, coriaceous-soft, separable, with the margin whitish, finally narrowly reflexed on the upper side and tomentose, or with margin everywhere free and curved outward; hymenium wood-brown, dry, usually bearing tubercules singly or in small clusters, with pale tips; basidia longitudinally septate, clavate, 25–36×9 μ, arranged between paraphyses with brown tips; spores simple, colorless, cylindric, curved, 15–16 × 6 μ.

Fructifications range up to 6 cm. long by 1–2 cm. wide and may be larger by confluence, about 700 μ thick; tubercules about $\frac{1}{2}$–1 mm. long.

Alabama. On bark of dead *Populus trichocarpa,* Idaho, and Oregon. July to September.

This species is distinguished by having a hymenium with configuration of a *Radulum* and cruciate basidia. The tubercules are often simple and cylindric, sometimes deformed and multifid. The wide distribution and yet the extremely local occurrence of this species together with the absence, until recently, of observations on its basidia have resulted in a very interesting synonymy. It is remarkable that this species, which occurs on *Fraxinus, Populus,* etc., in several countries of Europe, should have been collected in the United

States in Alabama, Idaho, and Oregon only. I am greatly indebted to Mr. L. Romell for a preparation from the type of *Radulum spinulosum* which makes possible the reference to this species.

Specimens examined:

Sweden: Stockholm, *L. Romell, 327,* and three unnumbered collections.

Alabama: *Peters,* Curtis Herb., 4543, preparation from type (in Kew Herb.).

Idaho: Kaniksu National Forest, Priest River, *J. R. Weir, 55.*

Oregon: Eugene, *C. J. Humphrey, 1103.*

5. E. gelatinosa Murrill, n. sp. Plate 27, fig. 12.

Type: in N. Y. Bot. Gard. Herb. and in Burt Herb.

Fructification coriaceous, effuso-reflexed, white when young, finally clay-colored, tomentose, soft to the touch, margin obtuse; context soft, spongy, zonate; hymenium tough, gelatinous, drying Hay's brown, even; basidia longitudinally septate, 13×11 μ; spores simple, colorless, even, flattened on one side, $8–10 \times 6$ μ.

Reflexed portion of fructification $1\frac{1}{2}$–2 cm. long, $2\frac{1}{2}$ cm. wide, $\frac{1}{2}$ cm. thick.

On rotting wood in wet, wooded regions. Jamaica. December and January.

Only two collections of one fructification each were made. That of December 17 is a white, young specimen, with no basidia developed, which shows the general habit and early characters of the species, but would not have been determinable except for the later collection of January 12–14, which shows the darker coloration assumed at maturity. The thick, spongy, soft pileus of the mature fructification distinguishes this species from others known at present.

Specimens examined:

Jamaica: Troy and Tyre, Cockpit country, *W. A. Murrill & W. Harris,* N. Y. Bot. Gard., Fungi of Jamaica, 1087, type (in N. Y. Bot. Gard. Herb.), a portion in Burt Herb.; Blue

Hole, Priestman's River region, *W A. Murrill,* N. Y. Bot. Gard., Fungi of Jamaica, 180, immature specimen.

SEBACINA

Sebacina Tulasne, L. R. and C., Ann. Sci. Nat. V. **15**: 223–226. *pl. 10. f. 6–10.* 1872; Linn. Soc. Bot. Jour. **13**: 35. 1873; Brefeld, Untersuch. Myk. **7**: 102–106. *pl. 6. f. 22–26.* 1888; Patouillard, Essai Taxon. Hym. 24, 25. 1900 (with the exclusion of section *Hirneolina*).—*Exidiopsis* Brefeld, Untersuch. Myk. **7**: 94. *pl. 5. f. 20–22.* 1888.—*Stypella* Möller, A., Bot. Mitth. a. d. Tropfen. 8, Protobasidiomyceten 166. *pl. 4. f. 6, 7.* 1895.

Fructification coriaceous, membranaceous or floccose, gelatinous, waxy or pulverulent, resupinate, with habit of *Corticium;* basidia longitudinally septate, close together or scattered, sometimes between bushy conidiophores; spores colorless, producing in germination a similar spore or a cluster of conidia.

The type species of the genus is *Corticium incrustans* Pers.

Sebacina incrustans occurs sometimes on the ground and incrusting herbaceous stems and various erect objects but is often on decaying wood; *S. Helvelloides* occurs on the ground and incrusting erect objects; *S. chlorascens* has been observed incrusting the mossy bases of living trees; the other species have been recorded only on dead wood and bark. A few members of this genus are thick and spongy and were originally included in *Thelephora;* usually the species are thin and *Corticium*-like in general habit and were in several instances published under *Corticium.* In the dried conditions some species of *Sebacina* may be tentatively recognized as such by having the hymenial surface glassy or resembling dried cartilage; but such a separation from *Corticium* is very uncertain, for some species of *Sebacina* dry with a dull, soft surface and some true Corticiums assume the appearance of dried cartilage in drying.

It seems probable that it will always be difficult to determine resupinate species of *Hymenomycetes;* it is not possible to do so from the descriptions alone of the earlier botanists. European authors have recently been enlarging such descrip-

tions by giving spore characters, dimensions of basidia, cystidia, and hyphae, and the presence or absence of clamp connections. Such additional characters may often be obtained quickly by microscopic examination of a portion of the fructification which has been teased out and crushed down in dilute potassium hydrate solution; by these helpful additional characters, some species may be recognized with reasonable accuracy, but there are comparatively few such species. Structure in section of the fructification affords important characters for the identification of resupinate species. In practical work with these species, a microscopical mount of a sectional preparation of a type specimen is the next best thing for purposes of comparison to having the type itself.

My method of determining a resupinate specimen is to observe closely its general habit and characters, such as consistency, adnation, thickness, surface, margin, substratum, and color. Color is an important character when given in terms of an adequate color standard. The color which the specimens retain in drying is often the only color character available; it is more constant, fortunately, than is commonly appreciated, for it has to be the color factor in the comparison of herbarium specimens. The preliminary observation may suggest that the species is one of several of somewhat similar habit which may be of the same genus or of various genera. The sectional preparations, which are now made, may present (*a*) a uniform, homogeneous arrangement of similar hyphae from substratum to hymenium, (*b*) dissimilar hyphae or organs distributed uniformly throughout the whole fructification, (*c*) a layered, heterogeneous arrangement of various types with the layers more or less sharply differentiated from one another, (*d*) a stratose arrangement having the first stratum extend from the substratum to the upper surface of the first hymenium, the second stratum a repetition of the first and borne on the first, and so on. Under *a* there are characteristic varieties of structure, constant for each species, such as all the hyphae in erect position extending from substratum to hymenial surface, or all interwoven, or all procumbent, and there are also constant

differences in regard to whether the hyphae are crowded close together or are loosely arranged. Under *c,* a conspicuous example would be one in which the layer next to the substratum is composed of longitudinally arranged hyphae (that is, parallel with the substratum) crowded closely together; from this layer, a few branches might extend outward at right angles to the first layer and form a layer of loosely arranged, erect hyphae — the second layer; the hyphae of the second layer might branch abruptly at its outer surface and bear a compact hymenial layer. Some species invariably form a loosely interwoven layer next to the substratum, and on the surface of this layer form a dense hymenial layer, as, for example, *Sebacina incrustans, S. chlorascens,* and *S. Helvelloides.* Sterile fructifications may frequently be determined by their general characters and structure in section.

The preparations which reveal structure in section, give also spores, basidia, paraphyses, and other organs. From the combination of general characters, structure in section, and details of spores and noteworthy organs, the species becomes manifest. Our species of *Sebacina* are described in accordance with this method in the following pages.

Key to the Species

Fructifications on the earth, running up and incrusting the bases of living stems and trunks as well as dead objects 1
Fructifications confined to bark and wood of dead branches and trunks ... 2

1. Sometimes passing into branches or ascending flaps; hymenial layer drying warm buff, 60–150 μ thick; paraphyses densely crowded and somewhat interwoven or adglutinated ..... *1. S. incrustans*
1. Pileate branches drying cream-color with a glaucous tint, imbricated, the apices spiculose or fimbriate; hymenial layer drying vinaceous brown, 140–240 μ thick ..... *3. S. chlorascens*
1. Not forming free branches or flaps; hymenial layer 200–300 μ thick; paraphyses straight and rod-like; basidia 20–25×15 μ ..... *4. S. Helvelloides*
2. Fructifications white or whitish when dry 3
2. Fructifications not white 4
3. Hymenium composed of unbranched, flexuous, even-walled, deeply staining, clavate organs 40–45×6 μ, in addition to few-branched paraphyses and basidia ..... *5. S. Shearii*
3. Hymenium composed of paraphyses and basidia; fructification 300–400 μ thick; margin thick, not closely adnate to substratum ..... *6. S. macrospora*
3. Hymenium composed of basidia and paraphyses; fructification 50–150 μ thick, shining white at first; margin very thin and closely adnate .. *7. S. calcea*
3. Hymenium composed of basidia and paraphyses; fructification 200–300 μ thick, dirty whitish; hyphae incrusted in upper two-thirds of fructification; margin thin and closely adnate ..... *8. S. monticola*

4. Drying ochroleucous, basidia at or near the surface in tissue not sharply differentiated as a layer from tissue near substratum; much crystalline matter about 100 μ below surface. On *Alnus*, South Carolina ..... *9. S. scariosa*
4. Drying some variety of brown 5
4. Drying fuscous to black 6

5. Drying cacao-brown (testaceous of Saccardo's 'Chromotaxia'); separable from substratum; resembling *S. incrustans* but with margin soon detached and spores 6–7×4½–5 μ. On juniper, Alabama ..... *2. S. deglubens*
5. Blue-purple when fresh, drying tawny olive to Saccardo's umber where directly on the wood; adnate to substratum; 30–45 μ thick; basidia 7–10×6–8 μ; spores 6–7×3–5 μ ..... *10. S. podlachica*
5. Drying cinnamon-brown; adnate to substratum; 100–140 μ thick; scattered paraphyses with bushy-branched, brown tops rise 45–60 μ above the basidia. On *Magnolia*, Delaware ..... *11. S. cinnamomea*
6. Hay's brown when moist, drying fuscous, the margin pale cartridge-buff; separable from substratum; 500–600 μ thick. On *Populus*, Idaho ..... *12. S. adusta*
6. Drying blackish plumbeous; adnate to substratum; 150–200 μ thick, the margin indeterminate. On *Populus*, Washington ..... *13. S. plumbea*
6. Grayish when moist, drying dark mouse-gray and shining; adnate to substratum; 50–160 μ thick, the margin indeterminate. On very rotten wood, New England ..... *14. S. atrata*

1. Sebacina incrustans Pers. ex Tulasne, Ann. Sci. Nat. Bot. V. **15**: 225. *pl. 10. f. 6–10.* 1872; Linn. Soc. Bot. Jour. **13**: 36. 1873. Plate 27, fig. 13.

Corticium incrustans Persoon, Obs. Myc. **1**: 39. 1796.—*Thelephora incrustans* Persoon, Syn. Fung. 573. 1801; Fries, Syst. Myc. **1**: 448. 1821.—*Thelephora sebacea* Persoon, Myc. Eur. **1**: 155. 1822; Fries, Elench. Fung. **1**: 214. 1828; Hym. Eur. 637. 1874; Saccardo, Syll. Fung. **6**: 540. 1888.—*Corticium sebaceum* Massee, Linn. Soc. Bot. Jour. **27**: 127. 1891.—*Merisma cristatum* Persoon, Syn. Fung. 583. 1801.—*Thelephora cristata* Fries, Syst. Myc. **1**: 434. 1821; Hym. Eur. 637. 1874; Saccardo, Syll. Fung. **6**: 539. 1888.—*Sebacina incrustans* Tul. ex Bresadola, in part (Hym. Hung. Kmet.), I. R. Acad. Sci. Agiati III. **3**: 117. 1897.

Illustrations: Tulasne, *loc. cit.*—Persoon, Com. Fung. Clav. *pl. 4. f. 4;* Berkeley, Outlines Brit. Fung. *pl. 17. f. 6;* Brefeld, Untersuch. Myk. **7**: *pl. 6. f. 22–26.* Hennings in Engl. & Prantl, Nat. Pflanzenfam. (**I.** 1 **): 91. *f. 59 C, D;* Nees, System *pl. 34. f. 256 B;* Patouillard, Tab. Anal. Fung. *f. 155;* and Essai Tax. Hym. 25. *f. 17 a, b;* Soc. Myc. Fr. Bul. **5**: *pl. 7. f. 11.*—See Saccardo, Syll. Fung. **20**: 945 for references to some additional illustrations which I have not seen.

Type: authentic specimens of *Thelephora incrustans* and *Merisma cristatum* from Persoon in Kew Herb.

Fructifications coriaceous-fleshy, varied in form, creeping on the ground and ascending and incrusting small erect objects and forming little columns and free branches, the apices somewhat awl-shaped or fringed, or effused and resupinate on bark, whitish, drying warm buff; structure in section, 250–400 μ thick, (1) with a broad layer of very loosely interwoven rather stiff hyphae, 2–2½ μ in diameter, which divide above into fine branches and form (2) a densely interwoven layer about 60–150 μ thick with the basidia in the upper 40–90 μ among the very fine (1½ μ in diameter), densely crowded, somewhat interwoven filaments from the subhymenium; basidia longitudinally septate, ovoid or pyriform, 12–20 $\times$ 9–14 μ; spores colorless, simple, even, flattened on one side or curved, 12–14 $\times$ 6–8 μ.

Fructifications 5–6 cm. long, 2–5 cm. wide, ascending objects 2–5 cm.; pileate flaps, when present, ½–1 cm. long.

On the ground in woods and incrusting objects, and resupinate on logs. Canada to Louisiana and westward to Missouri. June to October. Common.

S. incrustans is the common incrusting *Sebacina* of Eastern North America. It may usually be recognized at sight by coriaceous-fleshy consistency, occurrence on earth and running up and incrusting living objects, and pallid color. The thinner hymenial layer, paraphyses less rod-like in form, and finer and thinner-walled hyphae of layer next to the substratum afford structural characters separating specimens of this species from those of *S. chlorascens* and *S. Helvelloides.*

I exclude from the synonymy of *S. cristata, Clavaria laciniata* of Bulliard's 'Hist. Champ.' **1**: 208. *pl. 415. f. 1,* because in the absence of authentic specimens and observations in regard to spores and basidia, it is not certain that *C. laciniata* Bull. is *Merisma cristatum.* Bulliard's figures represent quite as well an incrusting European fungus communicated to me by Bresadola under the name *Thelephora fastidiosa* (Pers.) Fr., which has simple basidia and colorless echinulate spores. This species is the *Thelephora cristata*

of Patouillard's 'Tab. Anal. Fung.' No. 559, and *Cristella cristata* of his 'Essai Taxon. Hym.' *f. 28.* Patouillard notes that *Clavaria laciniata* is a synonym of the species which he figures. Because of the uncertainty as to whether figures of *Thelephora cristata* by European authors represent the true *Merisma* [*Sebacina*] *cristatum* Pers. or the echinulate-spored *T. fastidiosa* (Pers.), I have refrained from citing any illustrations except that of Persoon, of whose species I have studied an authentic specimen.

Specimens examined:

Exsiccati: Ellis, N. Am. Fungi, 513.

The specimen in Thuemen, Myc. Univ. 2009, under the name *Thelephora sebacea,* collected in France, is *Thelephora mollissima* Pers.

Europe: authentic specimens of *Thelephora incrustans* and *Merisma cristatum* from Persoon in Kew Herb.

Sweden: sterile specimen determined as *Thelephora cristata* by E. Fries (in Fries Herb.); Stockholm, *L. Romell, 54.*

Canada: *J. Macoun, 5, 10.*

Quebec: Hull, *J. Macoun, 203, 313.*

Ontario: near Ottawa, *J. Macoun, 40* (in Can. Geol. Surv. Herb.); London, *J. Dearness.*

Maine: Portage, *L. W. Riddle.*

New Hampshire: Shelburne, *W. G. Farlow* (in Farlow Herb.).

Vermont: Middlebury, *E. A. Burt,* two collections.

Massachusetts: Williamstown, *W. G. Farlow* (in Farlow Herb.).

New York: Hudson Falls, *S. H. Burnham, 2* (in Mo. Bot. Gard. Herb., 43995).

Pennsylvania: *Michener, 5821* (in Curtis Herb.); Trexlertown, *W. Herbst.*

District of Columbia: Rock Creek, *C. L. Shear, 793.*

North Carolina: Asheville, *H. C. Beardslee, 03126.*

South Carolina: *Ravenel, 1619* (in Curtis Herb.).

Louisiana: St. Martinville, *A. B. Langlois, F, 2015;* the same locality and collector, (3022 in Lloyd Herb.); Baton Rouge, *Edgerton & Humphrey, 667.*

Ohio: *A. P. Morgan* (in Lloyd Herb., 2655, 2656); Cincinnati,

C. G. Lloyd, 4198; Loveland, *D. L. James* (in U. S. Dept. Agr. Herb.).

Wisconsin: Blue Mounds, *E. T. and S. A. Harper, 864, 879, 880;* Madison, *W. Trelease* (in Mo. Bot. Gard. Herb., 5145, 44779); *C. J. Humphrey, 2146* (in Mo. Bot. Gard. Herb., 44784).

Illinois: Riverside, *E. T. and S. A. Harper, 698.*

Missouri: Creve Coeur, *E. A. Burt* (in Mo. Bot. Gard. Herb., 44763).

2. S. deglubens (Berk. & Curtis) Burt, n. comb.

Corticium deglubens Berk. & Curtis, Grevillea **1**: 166. 1873.

Type: type and cotype in Kew Herb. and Curtis Herb.

Fructification resupinate, effused, coriaceous, separable, white beneath, drying about cacao-brown, the margin very narrow, white, byssoid, soon detached; structure in section 250–300 μ thick, (1) with a very loosely interwoven layer 180–200 μ thick, having hyphae $1\frac{1}{2}$–2 μ in diameter which branch and form (2) a very densely interwoven layer 80 μ thick with the basidia in the upper 30 μ, not quite reaching to the surface, among the very fine, densely interwoven filaments from the subhymenium; basidia longitudinally septate, 15×10–12 μ; spores colorless, simple, even, flattened on one side, 6–$7 \times 4\frac{1}{2}$–5 μ.

On juniper, Alabama.

This fungus has the same type of structure which is found in resupinate specimens of *Sebacina incrustans.* It differs from the latter in having the hymenium darker, all the spores found in a sectional preparation a little smaller, and the hyphae of the layer next to the substratum a little smaller and more flaccid than those of *S. incrustans,* and the margin was described as soon detached. These differences may be merely the variation from specific type of a single collection, or they may be those of a subspecies of *S. incrustans* which has taken on the saprophytic life on dead wood, prevalent for most species of *Sebacina.* Until other collections, referable to *S. deglubens* are made, the former view appears the more probable.

Specimens examined:
Alabama: *Peters,* Curtis Herb., 4557, type (in Kew Herb.).

3. **S. chlorascens** Burt, n. sp. Plate 27, fig. 15.

Type: in Mo. Bot. Gard. Herb. and in Farlow Herb.

Fructification coriaceous, drying cream-color with glaucous tint, effused, ascending and incrusting the mossy bases of trees and forming imbricated, free, pileate, sterile branches, the apices spiculose or fimbriate; hymenium gelatinous, drying vinaceous brown, occurring in somewhat scattered spots on the lower portions of the fructification; structure in section 800 μ thick, with (1) a broad, spongy layer next to the substratum of loosely interwoven, rather rigid, even-walled hyphae $2\frac{1}{2}$–3 μ in diameter, which bear (2) a sharply differentiated hymenial layer 140–240 μ thick, composed of rod-like paraphyses 2 μ in diameter, between which occur basidia throughout the outer 60 μ of the layer; basidia longitudinally septate, pyriform, 15–18×12 μ; spores simple, colorless, flattened on one side, 10–$10\frac{1}{2}$× 6–7 μ.

Fig. 1
S. chlorascens
Paraphyses,
basidium×540.

Ascending objects 2–4 cm., 1–2 cm. broad; free branches up to 5 mm. long.

On mossy bases of living trees. Florida. Autumn.

As shown by the figures in pl. 27, the pileate branches of *S. chlorascens* do not resemble those of *S. incrustans.* The structure in section is different in every detail from that of specimens of the latter species and approaches more closely that of *S. Helvelloides,* but the fructification is thinner than that of the latter, has smaller basidia and spores, and the basidia distributed from the surface to about 60 μ below the surface, and forms free pileate branches.

Specimens examined:
Florida: Cocoanut Grove, *R. Thaxter, 98,* type (in Mo. Bot. Gard. Herb., 43923, and in Farlow Herb.).

4. **S. Helvelloides** (Schw.) Burt, n. comb. Plate 27, fig. 14.

Thelephora Helvelloides Schweinitz, Naturforsch. Ges. Leipzig Schrift. **1**: 108. 1822; Am. Phil. Soc. Trans. N. S. **4**: 168. 1834; Fries, Elenchus Fung. **1**: 193. 1828; Epicr. 541. 1836–1838.—*Corticium Helvelloides* Massee, Linn. Soc. Bot. Jour. **27**: 153. 1891.—*Corticium basale* Peck, N. Y. State Mus. Rept. **43**: 69 (23). 1890.

Type: in Herb. Schw. and portions in Curtis Herb. and in Kew Herb.

Fructification coriaceous, spongy, effused, convex, closely adnate and incrusting, on ground in mosses and on bark at bases of living trees, at first whitish, drying honey-color to warm buff; structure in section, with (1) a very thick spongy layer next the substratum, of loosely interwoven, branched, rather rigid, even-walled, brownish hyphae, 3–3½ μ in diameter, which bear (2) a fertile layer 200–300 μ thick made up of great numbers of erect, straight, cylindric paraphyses 2 μ in diameter, between which occur the basidia at about 40–50 μ below the surface; basidia longitudinally septate, pyriform, 20–25 $\times$ 15 μ; spores colorless, simple, flattened or slightly curved on one side, 12–13 $\times$ 6 μ.

Fructifications 3–15 cm. long and wide, drying about ½–2 mm. thick to 9 mm. thick in type which covers a cushion of moss plants.

On ground and bark at bases of living trees. New York to North Carolina. July and August.

Specimens of this species have somewhat the habit of thick specimens of *Coniophora puteana* but are of very different structure. The abundant, erect, unbranched, cylindric paraphyses often 200 μ long which compose the greater part of the hymenium, and the large basidia are reliable characters for identifying *Sebacina Helvelloides* when sections are studied; the coarser and colored hyphae of the species give an additional character separating it from *S. incrustans* when the latter occurs strictly resupinate.

The type specimen is abnormal in thickness and ridged surface by running over and incrusting a bed of moss. The hanging rootlets referred to in the original description are

moss stems. The specific name is rather fanciful and misleading.

Specimens examined:

New York: Whitehall, *C. H. Peck,* type of *Corticium basale* (in Coll. N. Y. State); Alcove, *C. L. Shear, 1221.*

North Carolina: Salem, *Schweinitz,* type (in Herb. Schw., in Curtis Herb., and in Kew Herb.).

5. S. Shearii Burt, n. sp. Plate 27, fig. 16.

Type: in Burt. Herb., and in Shear Herb.

Fructification coriaceous, effused, dull white, drying pale olive-buff, cracked, the margin determinate, entire; structure in section, 140–200 μ thick, with (1) a broad and dense layer next to the substratum of longitudinally arranged, slightly brownish, even-walled hyphae 1½–2 μ in diameter, which branch and curve outward at a right angle and form (2) a fertile, less compact layer 60–75 μ thick of suberect, few-branched paraphyses 3 μ in diameter, of basidia at about 15–20 μ below the surface, and of scattered, even-walled, flexuous, cylindric-clavate organs—perhaps gloeocystidia—40–45 × 6 μ, not emergent above the surface; basidia longitudinally septate, pyriform, 15 × 9 μ, with sterigmata 18 × 3 μ; spores colorless, simple, curved, 9–15 × 4½–6 μ.

Fig. 2 S. Shearii. Paraphysis at left, organ×540.

Fructifications in crevices of bark at first, 2 × 1 mm., at length, by confluence, 7 cm. long, 1 cm. broad.

On dead *Berberis vulgaris.* District of Columbia. October.

This species is well characterized by the presence in the hymenial layer of flexuous, even-walled organs, which are either latex or gloeocystidia, and by the broad layer of longitudinally arranged hyphae which shows relationship to *Eichleriella,* although the margin is not distinctly free. A few small granules are present on the hymenial surface but I do not know that they are a constant character.

Specimens examined:

District of Columbia: grounds U. S. Dept. Agr., Washington, *C. L. Shear, 1238,* type.

6. **S. macrospora** (E. & E.) Burt, n. comb.

Corticium macrosporum Ell. & Ev., Torr. Bot. Club Bul. **27**: 49. 1900.

Type: in N. Y. Bot. Gard. Herb.; specimens from type collection in Lloyd Herb., and in Burt Herb.

Fructification coriaceous, appressed, thin, dull white, cracked, the narrow, white, cottony margin sometimes narrowly involute; structure in section, 300–400 μ thick, with (1) a very broad layer of longitudinally arranged and somewhat obliquely ascending crowded hyphae $1\frac{1}{2}$ μ in diameter, colorless next to substratum but brownish in upper part of layer, which pass into (2) the hymenial layer 60–100 μ thick, consisting of erect, bushy paraphyses and of basidia; basidia longitudinally septate, pyriform to subglobose, 15×9–12 μ; spores colorless, simple, flattened on one side or curved, $10\frac{1}{2} \times 4\frac{1}{2}$–$6$ μ.

Appearing at first in orbicular patches 3–5 mm. in diameter, at length confluent and up to 4 cm. long, $1\frac{1}{2}$ cm. broad.

On pine (*Pinus Strobus*) limbs. Ohio. September.

This species is near *Sebacina calcea,* but the single collection which has been studied seems distinct from the latter by the thick, determinate margin, sometimes free and slightly upturned, by the greater thickness of the fructifications, by the brown hyphae of the middle region, and by walls of hyphae not gelatinously modified as in *S. calcea.* A relationship to *Eichleriella* is manifest in the broad layer of longitudinally arranged hyphae and in the tendency of the margin to be slightly free. The original description gives this species as on *"Fraxinus?",* but the limbs are *Pinus strobus.* The spores are not exceptionally large; the specific name was probably based on immature basidia.

Specimens examined.

Ohio: Linwood, *C. G. Lloyd, 3113,* type collection.

7. **S. calcea** (Pers.) Bresadola, Fungi Tridentini **2**: 64. *pl. 175.* 1892. Plate 27, fig. 17.

Thelephora calcea Persoon, Syn. Fung. 581. 1801; Myc. Eur. **1**: 153. 1822.—*Thelephora calcea c. albido-fuscescens*

Fries, Elenchus Fung. **1**: 215. 1828.—*Thelephora acerina* forma *Abietis* Fries, Syst. Myc. **1**: 453. 1821.—*Corticium Abietis* (Fr.) Romell, Bot. Not. **1895**: 72. 1895.—*Xerocarpus farinellus* Karsten, Finska Vet.-Soc. Bidrag **37**: 139. 1882.

Illustrations: Bresadola, *loc. cit.;* Patouillard, Essai Taxon. Hym. 25. *f. 17b.*

Fructification effused, closely adnate, crustaceous, slightly pulverulent, shining white at first, at length darkening in the central portion from cartridge-buff to pale drab-gray, cracked, the margin much thinner and farinaceous; structure in section, 50–150 μ thick, (1) with hyphae next the substratum interwoven, 2 μ thick, the wall gelatinously modified, (2) hymenial layer 40–60 μ thick, composed of basidia and of paraphyses branched at the apex into very fine branches loaded with minute granules; basidia more abundant in the lower portion of the hymenial layer, longitudinally septate, 14×9 μ; spores colorless, simple, cylindric, curved, $8–12 \times 4–5$ μ.

Fig. 3
S. calcea.
Paraphyses×540.

Fructifications 3–9 cm. long, 1–3 cm. broad.

On bark and wood of dead branches of spruce, pine, hemlock, white cedar, oak, ash, elm, maple, and elder. Canada, northern New England, and New York to Georgia, and in Washington. March to January—perhaps throughout the year.

As good distinctive macroscopic characters this species has: chalky white color with central portions ashy; powdery surface under a lens; thinness on drying and margin still thinner, so that it appears mealy under a lens rather than membranous. The fine branches and granules at the tips of the paraphyses show best in lactic acid preparations; potassium hydrate solution has a solvent action here. I have not been able to study an authentic specimen of *Thelephora calcea* Pers. and accept Bresadola's conclusion on this point.

Specimens examined:

Exsiccati: Romell, Fungi Exs. Scand. 129.

Austria: *G. Bresadola.*

Sweden: *L. Romell, 58, 59;* Stockholm, *L. Romell,* Fungi Exs.

Scand. These specimens are under the name *Corticium Abietis.*

Norway: Christiania, *M. N. Blytt* (in Herb. Fries and determined by Fries as *Corticium calceum*).

Finland: Mustiala, *P. A. Karsten,* under the name *Xerocarpus farinellus.*

Canada: *J. Macoun, 30, 33.*

New Hampshire: Chocorua, *W. G. Farlow,* two collections.

Vermont: Middlebury, *E. A. Burt,* two collections; Ripton, *E. A. Burt;* Little Notch, Bristol, *E. A. Burt.*

New York: Alcove, *C. L. Shear, 1134, 1208;* Hague, *C. H. Peck, 10;* Clear Water, *G. F. Atkinson,* Cornell Univ. Herb., 5049.

Georgia: Tipton, *C. J. Humphrey, 177;* Savannah, *C. J. Humphrey, 5106* (in Mo. Bot. Gard. Herb., 15081).

Washington: Bingen, *W. N. Suksdorf, 695, 711, 763, 765, 864.*

8. **S. monticola** Burt, n. sp.

Type: in Mo. Bot. Gard. Herb.

Fructification coriaceous, resupinate, cracked, dirty whitish approaching pale smoke-gray, the margin closely adnate; structure in section 200–300 μ thick, with hyphae colorless, 3–4 μ in diameter, ascending obliquely from substratum to surface, densely crowded together, more interwoven and little incrusted in the lower third of the fructification, but more loosely arranged and heavily incrusted in the whole upper two-thirds, terminating in incrusted paraphyses which are either simple or 2–4-branched and with the hyphal body about $2\frac{1}{2}$ μ in diameter under the incrustation; basidia about 40 μ below the surface of the hymenium, longitudinally septate, 15–20 $\times$ 9–12 μ; spores simple, colorless, even, cylindric, straight or curved, 9–$10\frac{1}{2}$ $\times$ 5–$5\frac{1}{2}$ μ.

The portion of the fructification described is 5 cm. long, about $1\frac{1}{2}$ cm. wide.

On bark of log of *Picea Engelmanni,* altitude 8,500 ft., Pike's Peak, Colorado. August.

This species belongs in the group with *Sebacina calcea* and *S. macrospora;* it is distinguished from both of these by the

incrustation of its hyphae and by simpler paraphyses, which are either unbranched or with only about 2–4 branches not branching repeatedly and becoming so attenuated as to be nearly invisible except for the granules which they bear.

Specimens examined:

Colorado: Pike's Peak, *G. G. Hedgcock,* comm. by C. J. Humphrey, 2571, type (in Mo. Bot. Gard. Herb., 15157).

9. **S. scariosa** (Berk. & Curtis) Burt, n. comb.

Corticium scariosum Berk. & Curtis, Grevillea **2**: 3. July, 1873.—*Corticium secedens* Saccardo, Syll. Fung. **6**: 635. 1888.

Type: type and cotype in Kew Herb. and Curtis Herb., respectively.

"Forming a thin, oblong, membranous stratum, without any distinct border; hymenium pulverulent ochroleucous."

—Original description.

Structure in section 300–600 μ thick, with hyphae 2 μ in diameter, branched, very loosely interwoven, extending from substratum to basidia, with walls gelatinously modified, imbedded in jelly, much crystalline matter about 90–120 μ below the hymenial surface; basidia at or near the surface, longitudinally septate, pyriform to subglobose, 12–15 $\times$ 9–12 μ; no spores found.

On alder, South Carolina.

The type specimens of this species have the general habit of *Peniophora gigantea,* which they also resemble in being separable and in cracking and peeling up from the substratum, but they are more lemon-yellow in color than specimens of the latter species. The structure in section is distinctive and suggestive of that of *Eichleriella alliciens.* Authors have sometimes confused *Corticium scariosum* B. & C. with *Corticium scariosum* B. and Br., published from Ceylon a few months later in the same year. The types of these fungi are not of the same genus, the American specimens having longitudinally septate basidia.

Specimens examined:

South Carolina: Society Hill, *M. A. Curtis, 4916* (type and cotype in Kew Herb. and Curtis Herb.).

10. S. podlachica Bresadola, Ann. Myc. **1**: 117. 1903.

Type: in Bresadola Herb. and a portion in Burt Herb.

Fructification effused, closely adnate, described as "e pallido-caerulea caesio-hyalina," drying tawny olive to Saccardo's umber where directly on the wood; structure in section 30–45 μ thick, with hyphae 2 μ in diameter closely crowded together and rising obliquely from substratum to the surface; basidia in upper 15 μ of fructification among the hyphal filaments, longitudinally septate, pyriform, 7–10 $\times$ 6–8 μ; spores colorless, simple, even, curved, 6–7 $\times$ 3–5 μ.

Covering areas 5 cm. long, 2 cm. broad.

On decaying coniferous wood, Massachusetts; on decaying beech wood, Russian Poland.

The Massachusetts collection was noted as blue-purple when fresh; in some places algae coating the wood have been covered by the fructification and the modified color of this algal layer is seen through the dried fructification; where the fungus coats the wood directly, the color of specimens which have been several years in the herbarium is the tawny olive. The American collection agrees closely with that communicated by Bresadola.

Specimens examined:

Russian Poland: on beech wood, *Eichler,* comm. by Bresadola, part of type.

Massachusetts: on coniferous wood, *W. G. Farlow.*

11. S. cinnamomea Burt, n. sp. Plate 27, fig. 18.

Type: in Burt Herb.

Fructification effused, coriaceous, dry, closely adnate, drying cinnamon-brown, the margin determinate, thick, entire; structure in section 100–140 μ thick, with (1) a layer 10–30 μ thick next to the substratum of longitudinally arranged, densely interwoven hyphae 2–2½ μ in diameter, which bear (2) the hymenial layer composed of basidia at the lower side of the layer, and of loosely arranged, highly branched, bush-

Fig. 4
S. cinnamomea.
Paraphysis×540.

shaped paraphyses with brown branches of zigzag form, which rise 45–60 μ above the basidia and give the characteristic color of the hymenium; basidia 15–20 × 9–11 μ, longitudinal septation not positively made out; no spores found; paraphyses 75 μ long, trunk 1½–2 μ in diameter, sweep of branched top about 20 μ.

Fructification 4 cm. long, 1 cm. broad.

On limbs of dead *Magnolia glauca.* Maryland. December.

It is not certain that this fungus is a *Sebacina,* for none of its basidium-like organs show longitudinal septa, although in a very few there is arrangement of the protoplasm suggestive of such septation. The specimen is a little too immature for generic reference but is probably a young *Sebacina* in my opinion. The species is distinct from others in possible genera by cinnamon-brown color, paraphyses scattered as to trunks but with such brown, bushy-branched tops as to form a compact surface of the color stated.

Specimens examined:

Maryland: Takoma Park, *C. L. Shear, 1339,* type.

12. S. adusta Burt, n. sp. Plate 27, fig. 19.

Type: in Burt Herb.

Fructification broadly effused, coriaceous, separable from the substratum, Hay's brown when moist, drying fuscous, the margin pale cartridge-buff, fibrillose-fimbriate; structure in section, 500–600 μ thick, composed of densely interwoven and obliquely ascending hyphae 3 μ in diameter, the walls not gelatinously modified, which bear the basidia at the surface of the hymenium; basidia longitudinally septate, pyriform, 12–16 × 8–10 μ; spores colorless, simple, curved, 10–12 × 4–5 μ.

Fig. 5
S. adusta.
Basidia and
hyphae×540.

Fructification 12 cm. long, 4 cm. broad.

On decorticated trunk of *Populus trichocarpa.* Idaho. July to September.

In the single collection of this species which has been received the margin is everywhere closely applied to the substratum and shows no tendency towards becoming free or

reflexed, hence the species must be included in *Sebacina*. The distinguishing specific characters are easy separation as an unbroken membrane of the moist fructification from the substratum, thickness of fructification, and position of the basidia at the surface of the hymenium.

Specimens examined:

Idaho: Kaniksu National Forest, Priest River, *J. R. Weir, 12,* type.

13. S. plumbea Burt, n. sp. Plate 27, fig. 20.

Type: in Burt Herb.

Fructification effused, closely adnate, drying blackish plumbeous, pruinose, the margin indeterminate; structure in section, 150–200 μ thick, with (1) a broad layer next to the substratum containing much crystalline matter in the interspaces between the interwoven suberect hyphae $1\frac{1}{2}$–2 μ in diameter, the wall gelatinously modified, and (2) a hymenial layer about 60 μ thick consisting of basidia, and of hyphae which branch and form a densely interwoven hymenial surface; basidia about 30 μ below the surface of hymenium, longitudinally septate, pyriform, 15–18 $\times$ 10–13 μ; spores colorless, simple, even, curved, 13–15 $\times$ $4\frac{1}{2}$–6 μ.

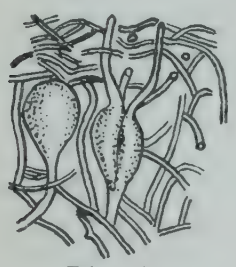

Fig. 6
S. plumbea. Basidia and hyphae×540.

Fructification 4–8 cm. long, $\frac{1}{2}$–1 cm. broad.

On blackened wood of *Populus trichocarpa*. Washington. November.

The coloration and habit of specimens of this species agree closely with those of the European *Corticium plumbeum* Fr. which have been received from Karsten, but the internal structure is wholly different from that of the latter.

Specimens examined:

Washington: Bingen, *W. N. Suksdorf, 862,* type.

14. S. atrata Burt, n. sp. Plate 27, fig. 21.

Type: in Burt Herb. and in Farlow Herb.

Fructification effused, somewhat gelatinous, closely adnate, grayish when moist, drying dark mouse-gray and shining, the margin thinning out and indeterminate; structure in section,

50–160 μ thick, with even-walled hyphae 3 μ in diameter, densely interwoven next to the substratum, then curving outward to form a hymenial layer 50–90 μ thick, consisting of erect, parallel, rod-like paraphyses 2 μ in diameter and of basidia about 30 μ below the surface of the hymenium; basidia longitudinally septate, pyriform, about 18 × 12 μ; spores colorless, simple, somewhat flattened on one side, 8–10 × 6–7 μ.

Fig. 7
S. atrata. Paraphysis, basidia × 540.

Fructifications 2½ cm. long, 1½ cm. broad.

On very rotten coniferous and frondose wood. New Hampshire and Massachusetts. May.

When bits of dried specimens of this species are moistened, they become softer and more gelatinous than is usual with those of other species of the genus, but walls of the hyphae do not show gelatinous modification in sectional preparations. The paraphyses are as noteworthy as those of *Sebacina Helvelloides,* being arranged close together side by side in a palisade layer. They are sometimes simple rods, sometimes divided into equal branches which rise side by side to the surface of the hymenium.

Specimens examined:

New Hampshire: Chocorua, *W. G. Farlow,* two collections (of which No. *a* is in Mo. Bot. Gard. Herb., 44782).

Massachusetts: Magnolia, *W. G. Farlow,* type.

(To be continued.)

Explanation of Plate

PLATE 26

The figures of this plate have been reproduced natural size from photographs of dried herbarium specimens.

Fig. 1. *Tremellodendron Cladonia.* *a*, from specimen collected in Canada by J. Macoun, 78; *b*, collected at Hague, New York, by C. H. Peck, 7; *c*, collected at Cincinnati, Ohio, by A. P. Morgan, Lloyd Herb., 32.

Fig. 2. *T. Cladonia*, from the type of *Thelephora gracilis*, collected in Alabama by F. S. Earle, 13.

Fig. 3. *T. candidum.* Collected at Newfane, Vermont, by C. D. Howe. *a* agrees closely with the type and is my standard for comparison; *b* could be separated without fracture into three portions, each having form of *a*.

Fig. 4. *T. merismatoides.* *a*, from specimen collected at York County, Pennsylvania, by N. M. Glatfelter; *b*, single fructification from the cluster *a*; *c*, from a very fasciculate specimen having stems grown together and branches still fimbriate at apex, collected at Haddonfield, New Jersey, by T. J. Collins.

Fig. 5. *T. simplex.* From type collected in Porto Rico, by J. R. Johnston. The fructification on the right is inverted.

Fig. 6. *T. pallidum.* *a*, from specimen collected at Middlebury, Vermont, by E. A. Burt; *b*, from specimen in Mo. Bot. Gard. Herb., 712370, collected at St. Louis, Missouri, by N. M. Glatfelter. Both show the growth together of the flattened pileate divisions.

Fig. 7. *T. tenue.* *a*, from type, collected at Chester Vale, Jamaica, by W. A. and E. L. Murrill, 400; *b*, from specimens collected at Cinchona, Jamaica, by the same collectors, 614.

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1 AND 2. TREMELLODENDRON CLADONIA.—3. T. CANDIDUM.—4. T. MERISMATOIDES.—5. T. SIMPLEX.—6. T. PALLIDUM.—7. T. TENUE.

Explanation of Plate

PLATE 27

The figures of this plate have been reproduced natural size from photographs of dried herbarium specimens, except in the cases noted otherwise.

Fig. 8. *Eichleriella Schrenkii.* From the type collected at San Antonio, Texas, by H. von Schrenk. *a*, photograph of a piece of limb bearing many fructifications, and *b*, drawing of median longitudinal section of single fructification, × 16.

Fig. 9. *E. Leveilliana.* From specimens collected at San Antonio, Texas, by H. von Schrenk.

Fig. 10. *E. alliciens.* From specimen collected at San Diego de los Baños, Cuba, by Earle and Murrill, 405, in part.

Fig. 11. *E. spinulosa.* From specimen collected at Priest River, Idaho, by J. R. Weir, 55.

Fig. 12. *E. gelatinosa.* From specimens collected in Jamaica by W. A. Murrill and W. Harris. *a*, upper surface of No. 180; *b*, type specimen, 1087, split longitudinally to show thickness of pileus and structure.

Fig. 13. *Sebacina incrustans.* *a*, from specimen collected at Middlebury, Vermont, by E. A. Burt; *b*, from specimen with pileate flaps, collected at Asheville, North Carolina, by H. C. Beardslee, 03126.

Fig. 14. *S. Helvelloides.* From specimen collected at Alcove, New York, by C. L. Shear, 1221. *a* shows upper surface; *b* is a vertical section from the same fructification to show thickness.

Fig. 15. *S. chlorascens.* From type specimen collected at Cocoanut Grove, Florida, by R. Thaxter, 98.

Fig. 16. *S. Shearii.* From type specimens collected at Washington, District of Columbia, by C. L. Shear, 1238.

Fig. 17. *S. calcea.* From specimen on white cedar bark, collected at Middlebury, Vermont, by E. A. Burt.

Fig. 18. *S. cinnamomea.* From type specimen collected at Takoma Park, Maryland, by C. L. Shear, 1339.

Fig. 19. *S. adusta.* From type specimen collected at Priest River, Idaho, by J. R. Weir, 12.

Fig. 20. *S. plumbea.* From type specimen collected at Bingen, Washington, by W. N. Suksdorf, 862.

Fig. 21. *S. atrata.* From specimen collected at Chocorua, New Hampshire, by W. G. Farlow.

BURT—THELEPHORACEAE OF NORTH AMERICA

8. EICHLERIELLA SCHRENKII.—9. E. LEVEILLIANA.—10. E. ALLICIENS.—11. E. SPINULOSA.—12. E. GELATINOSA.—13. SEBACINA INCRUSTANS.—14. S. HELVELLOIDES.—15. S. CHLORASCENS.—16. S. SHEARII.—17. S. CALCEA.—18. S. CINNAMOMEA.—19. S. ADUSTA.—20. S. PLUMBEA.—21. S. ATRATA.

ENZYME ACTION IN THE MARINE ALGAE

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In a previous contribution from this laboratory[1] attention has been called to the difficulties experienced in demonstrating enzyme action in *Fucus vesiculosus*. Because of the negative results there obtained it was deemed worth while to extend the study to certain representative forms of the three great groups of marine algae, the "greens," the "browns," and the "reds"; first, to ascertain whether this apparent inactivity were generally characteristic of the algae, and second, because of the light such an investigation might shed upon the general metabolism of the group.

Historical

Knowledge concerning enzyme activity and the distribution of enzymes in the algae is extremely meagre. The few papers that have found their way into the literature have been, for the most part, by-products of other studies and as such have dealt merely with isolated phases of the subject. From time to time, previous to actual demonstration, the presence of enzymes has been suggested by the work of various investigators. Arber ('01), attacking the problem of carbon assimilation in *Ulva latissima*,[2] found that the accumulation of starch in the tissue disappeared very slowly when the plant was subjected to darkness. This would suggest the presence of a diastase acting slowly. Spargo ('13) observed that *Chlamydomonas* began growth more slowly when the medium contained sucrose as a source of carbon than when dextrose was supplied. She suggests that the sugar is probably assim-

[1] Duggar, B. M. and Davis, A. R. Enzyme action in Fucus vesiculosus. Ann. Mo. Bot. Gard. 1:419–426. 1914.

[2] The binomials used throughout the historical review are those employed by the original investigators, no attempt being made to have them conform to any different existing nomenclature.

ilated in the hexose form and that sucrose must be split by invertase before becoming available. It is a well-known fact that diverse fresh-water algae can be grown in pure culture on media where asparagin and peptone are sources of nitrogen. It is hardly conceivable that the large protein molecule is assimilated directly and, *a priori,* this would argue for the presence of both an ereptase and a desamidizing enzyme.

ENZYMES FOUND IN THE MARINE ALGAE

Few workers have demonstrated enzymes present in either the fresh- or salt-water algae. Fischer ('05), working on the storage carbohydrates of *Anabaena* and *Oscillatoria,* found that the specific carbohydrate involved, which he named anabaenin, disappeared when the algal tissue was autolysed at 40°C. Microchemical tests showed glycogen split off. The action here, if it be due to ferments of the alga, is interesting in that the action was inhibited by .1 per cent acetic acid, by 1 per cent carbolic acid, and still more strangely, by concentrations of ethyl alcohol as low as 5 per cent. One per cent carbolic acid is quite often used as an antiseptic in enzyme experimentation, and the resistance of enzymes to even high concentrations of alcohol is common knowledge. No attempt was made to isolate the enzyme or to carry on experiments outside the cell.

Teodoresco ('12) found that *Chlamydomonas* in pure culture gave rise to an extracellular enzyme that decomposed sodium nucleate with the liberation of phosphorus. Later, ('12[a]) he demonstrated nucleases present in certain "blue-greens," "browns," and "reds." Unfortunately, differences in methods do not permit a true comparison of activity with that of the nuclease isolated by Dox ('10) from *Penicillium camemberti,* nor with that determined by Zaleski ('07) in the growing tips of *Vicia faba,* yet even a crude comparison is interesting. Dox added 2 grams of mold powder to 100 cc. of a 2 per cent solution of yeast nucleic acid, and maintaining his flasks at a temperature of 35–37°C. for forty-five days, found 51 milligrams of phosphorus (calculated as phosphoric acid) liberated. Teodoresco used a .5 per cent solution of sodium

nucleate with an unstated amount of crushed seaweed. The temperature during the incubation period varied from 21 to 26°C. for the different forms used. The following are his results for 100 cc. of substrate:

TABLE I

Alga	Days	Phosphorus as P_2O_5 mgms.
Cladophora frusta	57	54.3
Ceramium rubrum	51	76.6
Griffithsia setacea	37	62.5
Phormidium sp.	15	90.0

Zaleski crushed growing tips of *Vicia faba,* added water and an antiseptic, and allowed this material to autolyse at 34°C. for 4 days. At the end of that time the control flask showed a free phosphorus content of 13.6 milligrams and the one containing the active enzyme 51.2 milligrams. We have no means of knowing even the relative amount of enzyme present in any of these experiments and yet it seems that the algal nuclease compares very favorably with that isolated from the fungi and the higher plants.

The classes in plant physiology at the Marine Biological Laboratory, Woods Hole, for several years past have qualitatively determined diastase in *Ulva lactuca.* Bartholemew ('14), working on the question of starch in the *Florideae,* conclusively demonstrated diastase present in such "reds" as *Polysiphonia variegata, Dasya elegans, Agardhiella tenera,* and *Ceramium sp.* In order to isolate the enzyme, he used the ordinary method of precipitation by alcohol from an aqueous extract of crushed tissue. Starch as paste was hydrolysed rather slowly to an undetermined reducing sugar, presumably dextrose, 5 cc. of .25 per cent starch paste with a relatively large amount of the enzyme material requiring from 6 to 9 days for the completion of hydrolysis. Microscopic observation of the attacked starch grain showed corrosion similar to that caused by the translocation diastase of the barley. Torup (Krefting and Torup, '09) had previously isolated an enzyme from fresh *Laminaria* that hydrolysed the characteristic storage carbohydrate of that alga, laminarin, to dextrose.

Atkins ('14) investigated the oxidases and peroxidases of twenty-nine diverse algae. Using guaiacum as a reagent, oxidases were demonstrated in but one—*Furcellaria fastigiata*—while peroxidases were shown present in seven. Alpha naphthol gave negative reactions for all the forms studied, while with it peroxidases could be determined in but two—*Delesseria sanguinea* and *Furcellaria fastigiata*. He calls attention to the reducing power of the tissues of certain algae and suggests that such agents may be responsible for the failure to obtain positive tests in the other forms. Reed ('15, '15[a]), on the other hand, holds that many of these algae may show a specific oxidative ability. Like Atkins, he found that the ordinary reagents, such as gum guaiac, alpha naphthol, and aloin, gave negative results in all but one or two instances. When, however, alpha naphthol and para-phenylenediamine, para-phenylenediamine alone, or the hydrochlorides of these two were used in the presence of peroxide, positive tests were very generally obtained.

As earlier indicated, the results obtained by Duggar and Davis ('14) for *Fucus vesiculosus* were very generally negative. This was true even though a great variety of substrates were used under varying conditions, and only vigorously growing plants, fresh crushed, or dried and powdered, were employed for enzyme action. The results are exceedingly difficult to explain. It might well be that the enzymes were present but in such small amounts as to escape detection by the ordinary methods. Methods of enzyme isolation are still crude and they undoubtedly involve some loss of the ferments. Another factor suggested in the preliminary paper, was that the death of the cell might liberate certain substances which would then be free to unite with the enzyme complex, throwing it out of the sphere of action.

SOME STORAGE PRODUCTS OF THE ALGAE

It is often assumed that the presence of storage products in the plant is generally linked with the presence of specific enzymes—starch with diastase, inulin with inulase, fats with lipase, hemicelluloses with cytase, etc. These enzymes may be present at all times, as the diastase of the potato tuber and

the diastase and maltase of the barley grain, or they may only arise when there is food transformation and translocation, as in germinating seeds. However, in the light of such possibilities of association, it is worth while to call attention briefly to some of the work that has been done on the chief storage products of the algae.

The carbohydrates have been more worked over in this respect than has any other chemical group, but much confusion still exists regarding their exact status in assimilation. Much of the study has been on the cleavage products, obtained by acid hydrolysis, of undetermined carbohydrates. These, however, are not a true index of the distribution and more restricted chemical nature of assimilable carbohydrates in the living plant; one must look rather to the work of those who have limited themselves to the isolation and determination of unaltered carbohydrates.

CHLOROPHYCEAE

Polysaccharides.—Nägeli ('63) reported "sphärokristalle" in *Acetabularia* which Leitgeb ('87) later showed were inulin. The former worker also demonstrated the presence of this carbohydrate in various members of the *Dasycladaceae.* Küster ('99) has more recently found characteristic crystal formations in *Derbesia* and *Bryopsis* which, from the many reactions they gave, appear to have been inulin. Famintzin ('67) and Krause ('70) worked on the effect of light on starch formation in *Spirogyra,* and within recent years, Timberlake ('01) has contributed observations on the starch of *Hydrodictyon.* Oltmanns ('05, p. 147) speaks of starch accumulation in the *Conjugales, Volvocales, Ulotrichales, Charales, Siphonocladiales,* and some of the *Siphonales.* He considers it the first visible product of assimilation, but thinks that it may also function as a reserve. Starch in the marine forms seems to be quite widely distributed. In the work of Arber ('01), to which reference has already been made, starch accumulation in the tissues of *Ulva, Cladophora,* and *Enteromorpha* was easily demonstrated by means of iodine. Swartz ('11) isolated starch from *Ulva* but was unable to prove its presence in *Enteromorpha,* a closely re-

lated genus. She concluded that the carbohydrates existed in the form of hemicelluloses, probably as pentosans.

Glycogen, although frequently found in the "blue-greens," where, as held by some authors (Fischer, '05), it functions as the chief reserve carbohydrate, has been demonstrated in but one case, as far as is known, in the *Chlorophyceae,* and that by Beyerinck ('04) in *Chlorella variegata.*

Simple sugars.—The nature of the simple sugars in the group is indefinite. Klebs ('96) reported a substance in the cells of certain *Heterokonteae* that reduced Fehling's solution, but this means little since most algae contain non-carbohydrate reducing substances made up chiefly of tannins and tannoidal bodies. Tihomirov ('10) used the phenylhydrazine method as modified by Senft ('04) for the detection of osozone-forming sugars in algal tissues in this group, chiefly those of *Codium bursa* and *C. tomentosum.* After a period of thirty days, for these two forms, yellow amorphous deposits appeared in the cells indicating a sugar reaction. The definite sugars these osozones represented could not be determined, but he suggests the possibility of dextrose and d-galactose. It seems evident that they must be present in very small quantities in the tissues investigated.

PHAEOPHYCEAE

Polysaccharides.—Starch is conspicuously absent from the great group of "browns," but there are, however, certain less highly condensed polysaccharides present. Schmiedeberg ('85) speaks of a dextrin-like compound which he isolated from *Laminaria.* He gave to it the name "laminarin" and the general formula, $10(C_6O_{10}O_5)$-$9H_2O$. There seems, however, to be some confusion regarding his method of arriving at these figures. Torup ('09) was able to extract a dextrin from *Laminaria sp.* with warm water, that gave dextrose on hydrolysis. This could be isolated only during the winter months. He called it "kreftin." Kylin ('13), extracting crushed *Laminaria saccharina, Fucus vesiculosus,* and *Ascophyllum nodosum,* obtained a dextrin-like compound similar to that described by Schmiedeberg and he retained Schmiede-

berg's name, "laminarin." He showed also that Torup's "kreftin" was without doubt a modification of "laminarin." Kylin ascribes to "laminarin" the same physiological function that starch performs in the higher plants, i. e., that of a reserve product. In a more recent paper ('15) he shows that there is an accumulation of the "laminarin" in the tissues of the algae during the summer months, while during the winter and spring this reserve is drawn upon by the young fronds until by the end of March very little of it is demonstrable.

Kylin was also able to clear up much of the confusion that has attended observation of the light-refracting granules present in the cells of many members of the group. They had been variously considered as of fatty nature, proteinaceous, tannin-like, and glucosidal. Reinke ('76) demonstrated fat-like bodies in the cells of *Fucus* that he looked upon as the first visible products of assimilation, a point of view later supported by Hansen ('93). Schmitz ('83) claimed two distinct bodies present, one of which, although it did not react with iodine, he called "phaeophyceenstärke," the other giving the ordinary reactions for fats. Hansteen ('92) had observed bodies in the same plant which he maintained were of carbohydrate composition and to which he applied the term, "fucosankörner." Crato ('92, '93), the same year, investigating the fat globules observed by Schmitz, suggested that they were either phloroglucin or a derivative of it, since they colored red with vanillin-hydrochloric acid. This conception was held by Bruns ('94) as well. In a later paper, Hansteen ('00) observed that the "fucosankörner" were formed in the presence of light, and this to his mind indicated that they function as the first assimilable products. Hunger's ('02) work two years later pointed to Hansteen's "fucosankörner" as being glucosidal in nature, the carbohydrate attached being bound up with phloroglucin, or at times, with tannic acid. Some of the larger "körner" gave fat reactions, some protein. Kylin found three definite bodies in the cell, the nature of which had been confused by earlier workers—fat globules, proteinaceous particles, and tannin-like bodies—these latter probably representing the "fucosankörner" of

Hansteen. He holds that none of these are to be considered the first visible products of assimilation, and suggests that here, as in most phanerogams, carbohydrates function in that rôle.

Simple sugars.—As far as is known, Tihomirov ('10) was the first to definitely demonstrate simple sugars in these plants. He used the same phenylhydrazine method employed with the "greens," but as was the case there, was unable to connect the osozones with definite sugars. The osozones took considerable periods of time to form, in some cases as long as five months, evidence pointing to the low concentration of sugars in the cell. It is a question, too, whether during this long period of incubation some of the more highly condensed carbohydrates in the cell were not hydrolysed far enough to give the sugar tests. Using the same method, Kylin ('13) was unable to substantiate these results. However, by using 40 per cent alcohol as an extracting agent, precipitating the inorganic material with lead acetate, and then purifying with alcohol, he was able to obtain reducing sugars from several of the *Fucoideae,* particularly *Laminaria digitata, L. saccharina, Ascophyllum nodosum,* and *Fucus vesiculosus.* In all cases Seliwanoff's test for fructose was positive, while dextrose was demonstrated by its osozone. These sugars he considers the first products of assimilation referred to above.

RHODOPHYCEAE

Polysaccharides.—The so-called Florideae-starch has been the source of many investigations, from the time of Nägeli ('58) and Van Tieghem ('65) to the present day. Although not identical perhaps, it is very similar to the starch of the higher plants, and as very generally held, it undoubtedly functions in the same manner. Meyer ('95), Kolkwitz ('00), and Bartholemew ('14) hold the opinion that it represents a combination between true starch and dextrin, while Bütschli ('03) suggests the possibility of its being a transitional stage between amyloporphyrin and amyloerythrin. Kylin ('13) considers it as standing midway between starch and dextrin. This investigator succeeded in isolating

Florideae-starch from *Furcellaria fastigiata,* that was readily hydrolysed to dextrose by malt diastase, and it will be remembered that Bartholemew ('14) isolated diastase from several of the "reds" that split phanerogamic starch to reducing sugars.

Simple sugars.—Very little work has been done on the di- and monosaccharides of the "reds." Tihomirov ('10) succeeded in obtaining the same yellow amorphous osozone deposits in the tissues of *Sphaerococcus crispus* and *Gigartina mamillosa* that he had in certain members of the "greens" and "browns," but here, as in the other groups, the specific osozone involved could not be determined.

FATS AS STORAGE PRODUCTS

Many observations have made it evident that fats in some form or other are generally present in the algae, their peculiar rôle, however, having been very little investigated. In some of the siphonaceous forms, particularly *Vaucheria,* they seem to replace carbohydrates. Whether fats are to be regarded as the first visible products of assimilation in these forms is disputed. Some workers hold them to be reserve products, some by-products of metabolism. If they are utilized as a reserve or storage product in any of the forms, one might expect to find evidences of lipolytic action, yet none has been reported so far.

As stated by Czapek ('13, p. 761), Loew and Bokorny find that *Spirogyra* and other filamentous forms contain 6 to 9 per cent of the dry weight as fat. This probably includes lecithin. The same authority gives the following results as obtained by Sestini, the figures being percentages of the dry weight:

Vaucheria pilus	2.94
Ulva latissima	.21
Fucus vesiculosus	.67
Valonia aegagropila	.15
Gracilaria confervoides	.11

König and Bettels ('05) made a large number of analyses of the dry tissues of a variety of marine algae and found a fat

content ranging from .20 per cent in *Enteromorpha* to .98 per cent in *Porphyra.*

RELATION OF THE ALGAE TO NITROGEN

Some of the recent work on pure culture methods with fresh-water algae, such as that of Beyerinck ('90), Charpentier ('03, '03[a]), Chick ('03), Artari ('13), Spargo ('13), and Schramm ('14) have conclusively proved that these forms can utilize organic nitrogen. Furthermore, the work of Letts and Hawthorne ('11), and Foster ('14) point to the fact that the marine forms may have this capacity as well. Letts and Hawthorne and also Letts and Richards ('11) showed that *Ulva latissima* grew better in sewage-contaminated sea-water than in water from the open sea. Foster placed strips of *Ulva lactuca* in normal and artificial sea-water, containing in addition compounds of nitrogen in varying concentrations. When urea or ammonium sulphate was added to either solution an accelerated growth took place.

The current conception concerning the assimilation of organic nitrogen by the animal organism is that the protein and amino acid molecule must be completely desamidized before the building-up process can begin. In the absence of definite information to the contrary, we can conceive of a parallel situation existing in the plant. The question at once arises in regard to the algae, whether this be due to the agency of amidases formed by the tissue, or to the activity of desamidizing bacteria, the presence of which Brandt ('99), Gran ('02), Baur ('02), Reinke ('03), Benecke and Keutner ('03), and others have shown to exist abundantly in harbor waters. Neither Letts and Hawthorne nor Foster worked with pure cultures, and these bacteria may have been the agency in their experiments to render the amino-nitrogen assimilable.

CARBOHYDRATES AND CARBOHYDRATE CLEAVAGE PRODUCTS OF ALGAL SLIME

Besides the carbohydrates that may be directly assimilable, we find those whose function in metabolism is more or less disputed. The so-called algal slime is made up chiefly of such products.

Chemical composition.—Greenish ('81) found agar from *Fucus amylaceus* to consist of 37.21 per cent gelose (probably galactan since it passed to galactose on hydrolysis) and that from *Sphaerococcus crispus* of 60 per cent of the same carbohydrate. König and Bettels ('05) give the carbohydrate composition of agar-agar from *Gelidium* as 33 per cent galactans and 3.1 per cent pentosans; by hydrolysis, d-galactose and levulinic acid were split off. Günther and Tollens ('90) found fucosan in *Fucus* from which the methyl-pentose, fucose, was split off. Galactose was also demonstrated. Sebor ('00) obtained galactose, glucose, and fructose from the slime of *Chondrus crispus* by acid hydrolysis. He held that the slime is a very complex carbohydrate of high molecular weight, made up chiefly of galactosan, glucosan, and fructosan.

The cleavage products of *Porphyra laciniata,* as investigated by Oshima and Tollens ('01), were found to consist chiefly of l-galactose and mannose, but glucose, fucose, and other pentoses were also obtained. Müther and Tollens ('04) found methyl-pentosans in several of the *Fucaceae.* König and Bettels ('05), working on the carbohydrate hydrolytic products of various species of *Porphyra, Gelidium, Laminaria, Cystophyllum,* and *Enteromorpha,* found them to consist of such hexoses as galactose, dextrose, and fructose, as well as several pentoses, chiefly methyl-pentoses. *Enteromorpha* yielded a pentose—rhamnose. The results of Swartz ('11) agree with those above, namely, that for all forms studied, representatives of the "greens," "browns," and "reds," pentosans were always present, and galactans frequently so. Kylin ('13), by direct extraction with warm water of crushed *Ceramium, Furcellaria,* and *Dumotia,* obtained substances that gave the mucic acid test for galactose, as well as the phloroglucin test for pentosans. Substances giving pentosan reactions alone were isolated from the slime of *Ascophyllum nodosum, Fucus vesiculosus,* and *Laminaria sp.* He was apparently unable to substantiate the finding of galactan in *Fucus* by Günther and Tollens, and this negative result also conflicts with the statement of Swartz, who says that the gelatinization in the algae is due to the galactan groups.

Kylin ('14) and others have also demonstrated pectin-like compounds forming the middle lamella in various members of the *Fucaceae.* These exist as the calcium salts of pectic-like acids which Kylin designates "Fucinsäure" and "Alginsäure."

PHYSIOLOGICAL SIGNIFICANCE OF ALGAL SLIME

It is seen that algal slime is made up chiefly of the anhydrides of hexoses and pentoses—carbohydrates that must be broken down to simpler form before assimilation by the plant would be possible. Two questions naturally arise: (1) Do the algae concerned form enzymes that will hydrolyse these highly condensed carbohydrates to assimilable form? (2) Does the slime itself arise through the breaking down of the hemicelluloses of the cell wall through enzymic or other causes, or does it represent a final stage in the condensation of those hemicelluloses?

Algal slime as a reserve product.—Galactanases and mannases have been demonstrated in the phanerogams and in the fungi by Bourquelot and Hérissey ('99), Grüss ('02), and Hérissey ('03). The last worker especially has clearly shown the distinct rôle that galactans and mannans may play as reserve products in the tubers of the *Orchidaceae* and in many of the *Leguminoseae.* It is significant that Gran ('02[a]) was able to isolate a marine bacillus, *B. gelaticus,* that acted on part of the constituents of agar-agar to give a reducing sugar. From the standpoint of a possible symbiosis it would be interesting to know if this organism has the ability to fix free nitrogen. Saiki ('06) experimented with a number of algal and lichen preparations containing a large proportion of carbohydrates as galactans and pentosans, and concluded that the latter could not be transformed into sugars readily by carbohydrate digesting enzymes of animal origin and scarcely more so by the vegetable enzymes, either of the higher plants or of bacteria.

Still less is known of the digestion of pentosans by the higher plants. Schöne and Tollens ('92) found no decrease in the amount of pentosans during germination and conclude

that they cannot function as reserves. Cross, Bevan, and Smith ('95) consider the pentosans as by-products of metabolism and once formed remain unalterable. Ravenna and Cereser ('09), on the other hand, in some very interesting experiments, found that when dextrose was supplied as the sole nutrient to the leaves, pentosans increased greatly, especially in the light. If, however, the function of chlorophyll is inhibited, a decrease in the amount of pentosans takes place. These results form the basis for their conclusion that pentosans may sometimes function as reserves.

The origin of algal slime.—The question concerning the origin of the slimy and gummy constituents of cells, whether they arise through enzyme action or through other causes, has provoked much discussion. There is considerable doubt whether such gums can arise directly from true cellulose or whether they are, at least in the case of the plant mucilages, laid down as such.

One might roughly group the plant gums into those arising as a result of some external excitant, such as, for example, cherry gum, acacia gum, gums of citrus, etc., and those which seem to be normal constituents of the plant, as the mucilages found in the epidermis of many seeds and plant organs. The former arise as a result of a pathological condition; the latter, as far as we know, are normal physiological products and as such are more nearly comparable to the algal slime.

Klebs ('84), investigating slime formation in some of the lower algae, particularly some of the *Desmidiaceae,* held that it was not a conversion product of cellulose. Hauptfleisch ('88) substantiated the conclusion of Klebs, and going further, states that it arises in this particular case through the activity of the protoplasm, being excreted through pores. Oltmanns ('04, p. 76) illustrates very clearly the arrangement of these pores. Tschirsch ('89) differentiates these slimes or mucilages into those giving a cellulose reaction and those not doing so, the former having some relation perhaps to the cellulose, but the latter being laid down on the cell wall as such by the protoplasm. He holds the epidermal slime of *Spirogyra* to be of this latter type, which he calls "echter Schleim." In

the same work the author concludes the slime of the *Fucaceae* and of the *Florideae* to be of the "echter" type, occurring here, however, not as a layer laid down on the inner cell wall, but as an intercellular substance. Guignard ('93) held much the same view, and in an excellent histological investigation, clearly demonstrated the presence of slime or mucilage ducts in the *Laminariaceae.*

Mucilages very similar in nature and origin to the algal slimes occur in the higher plants, and much more work has been done with them than with those occurring in the algae. It is hardly necessary to go into the historical aspect of this phase of the work. The current conception of its origin is voiced by Walliczek ('93), who, investigating rather fully the location of different types of normal mucilages by means of suitable stains, found that in almost all cases they were laid down as such. According to him, the slime forms secondary layers on the cell wall which he designates "Membranverdickungsschichten"—layers that in many instances almost completely fill the cell. Where the epidermal layer of seeds becomes gelatinous, as, for example, in those of flax, mistletoe, various *Cruciferae,* etc., it is this inner cell wall which Walliczek holds to be the seat of slime formation. Upon contact with water the slime swells remarkably, filling the cell and at times even bursting it. There may or may not be an actual hydrolysis of the true cellulose, but if there is it seems rarely to enter into mucilage formation.

Experimental

Forms used.—The algae to be used for enzyme investigation were collected in the vicinity of Woods Hole, Massachusetts, during the summers of 1913–14, at which time the plants were also dried for winter work at the Missouri Botanical Garden. Work with the fresh tissue was carried on at the Marine Biological Laboratory, Woods Hole, during the latter summer. The selection of forms with which to work was limited to those relatively abundant in the neighboring waters, a further limiting factor in selection being relative freedom from adhering marine organisms. Only those plants

were selected that were "clean." This was an important precaution, since many adhering organisms have been found to be quite active enzymatically, and the presence of even a few might well lead to serious errors in the final results. The following forms lent themselves most readily to the work:[1]

Chlorophyceae

Ulva lactuca (L.) Le Jolis
Enteromorpha intestinalis (L.) Link

Phaeophyceae

Laminaria Agardhii Kjellm.
Ascophyllum nodosum (L.) Le Jolis
Mesogloea divaricata (Ag.) Kutz

Rhodophyceae

Ceramium rubrum (Huds.) Ag.
Agardhiella tenera (J. Ag.) Schmitz
Rhodymenia palmata (L.) Grev.
Chondrus crispus (L.) Stack.

Preparation of algal material.—In addition to the question of cleanliness, great care was taken to select only plants that were in a young, vigorously growing condition. These were brought into the laboratory, placed in large aquarium jars containing salt water, picked over, and all detectable foreign matter removed. A thorough washing in running salt water for two hours was then given, after which, with the exception of one or two forms that rapidly gelatinized, the plants were placed in running fresh water for 10 or 15 minutes. This fresh water treatment was very efficacious in causing small snails and other minute marine organisms to loosen their hold.

The plants so washed were either crushed and used at once with the substrate for enzyme action, or they were dried for future use. In either case, two general ways of using the ma-

[1] With the exception of *Laminaria Agardhii* and *Agardhiella tenera*, these binomials conform to the nomenclature as given by Farlow (Marine algae of New England, pp. 1–210. *pl. 1–14.* 1881); these two forms are as given by De Toni (Sylloge Algarum 3: p. 349. 1895) and Engler and Prantl (Nat. Pflanzenfam. 1^2:371. 1896), respectively.

terial for such action were employed. The tissue was added directly to the substrate, or it was extracted with water by the method to be described later and a water-diffusion used of the alcohol precipitate. If the fresh tissue were to be used directly, it was ground in a meat chopper two or three times, then pounded in a large mortar with an equal amount of fine, clean, quartz sand. This treatment gave a very homogeneous pulp, one in which a large number of the cells were broken down. If desired for future use, the plants were either dried at room temperature or dehydrated by the following modified Buchner "dauerhefe" process:

3 volumes	95 per cent alcohol	for 15 minutes.
3 volumes	acetone	for 15 minutes.
3 volumes	95 per cent alcohol	for 10 minutes.
3 volumes	acetone	for 5 minutes.
2 volumes	absolute alcohol or ether	for 5 minutes.

After each treatment, the dehydrating liquid was pressed out through two thicknesses of cheese cloth by making a tourniquet. Upon the removal of the absolute alcohol or ether, the tissue was spread out on adsorbent paper, either filter paper or paper toweling, until all the dehydrating agent had evaporated. A uniformly dry, brittle, easily crushed material usually resulted that was roughly broken up and stored in tightly stoppered bottles for future use. Those plants that were dried at room temperature were simply wrapped in paper or placed in paper bags until needed.

The crushing of the dry material was accomplished in the same manner as was the fresh. Usually it was ground twice or more in an ordinary meal mill, then pounded in a mortar with an equal weight of quartz sand until a very fine powder was obtained. The sand was dispensed with if the tissue were easily crushed.

Methods of isolating the enzymes.—As indicated above, there were two general methods of using the material for enzyme action: first, adding the crushed tissue directly to the substrate, either as fresh pulp or as "dauerhefe" powder;

second, by extracting the tissue with water and precipitating the protein-enzyme complex with several volumes of 95 per cent alcohol. Wherever possible the first method was used, since it was thought that in this way the maximum enzymic activity would be obtained. However, the fresh pulp and the powdered material contained a substance, or substances (probably tannoidal bodies), that reduced copper from Fehling's solution, and so in all experiments where sugar determinations were involved, it was found necessary to use the extraction and precipitation method; by this means all the unknown reducing substances were avoided. The method was as follows:

To a known amount of the crushed, fresh algal material, 3–5 volumes by weight of distilled water were added; to the powdered tissue, 8–10 volumes. The amounts varied owing to the differences in viscosity produced by the different algae. In some forms a relatively large amount of water was necessary in order to overcome difficulties in handling due to this high viscosity. Two per cent toluene was generally added as an antiseptic, or in some cases, 1 per cent chloroform-thymol mixture was used (5 per cent thymol dissolved in chloroform), and the extraction allowed to go for 12 hours at room temperature, or for 4 hours at 35° C. The water extract, if at all viscous, was then filtered off through two thicknesses of cheese cloth and the algal tissue pressed out as completely as possible by making a tourniquet of the cloth. Filtering through cotton was tried at first, both with pressure and without, but the method had the disadvantage of slowness and also that of adsorption by the cotton. Neither did filter paper lend itself efficiently to the filtration of such viscous liquids, a drier residue being obtainable in a shorter time by the cheese cloth-tourniquet method. A press would have been desirable but none was at hand. If the medium were not viscous, it was filtered with pressure through a thin layer of cotton or a coarse filter paper in the bottom of a Buchner funnel.

The protein-enzyme complex was precipitated with 3 volumes of 95 per cent alcohol. After a few moments the

coagulum either came to the top or settled to the bottom of the vessel—if to the top, it was usually very much aggregated and little difficulty was experienced in the filtering, if to the bottom, it was generally in a very finely divided condition and unless care was exercised in the decantation of the supernatant liquid the pores of the filter soon became clogged, resulting in extremely slow filtration. Time was therefore given for a complete settling out (15 minutes to half an hour sufficed) and all the clear fluid filtered off before the coagulum reached the filter paper.

A homogeneous diffusion of the precipitate was made by placing the filter paper with the attached coagulum in a known volume of distilled water. The paper could soon be removed without loss of material, and the weight of the original fresh or dry tissue represented by an aliquot portion of the solution easily reckoned. If the precipitate were not required immediately, it was dried on a filter paper at room temperature and stored in stoppered jars. In none of the experiments was the enzyme material purified further.

When dissolved in water, the precipitates behaved differently. Some, especially those where much slime had been noticed in the extraction, gave an extremely viscous suspension, others a suspension of low viscosity. In *Laminaria* and *Chondrus,* where the extract had been quite viscous and slimy, the protein was caught up in the precipitated slime in such a way as to make the freeing of it practically impossible. The precipitate in these cases was very large and when diffused in water gave a suspension difficult to handle. *Rhodymenia, Ceramium,* and *Enteromorpha,* on the other hand, gave a finely divided precipitate that produced no viscosity.

Glassware, antiseptics, solutions, etc.—With few exceptions, the various experiments were set up in 125 cc. Erlenmeyer flasks. All glassware was thoroughly cleaned with strong soap and then with chromic-sulphuric cleaning mixture, after which it was rinsed several times with tap and distilled water.

Solutions were made up from either Merck's or Kahlbaum's "guarantiert" chemicals.

Three general antiseptics were used—toluene, alcohol to 20 per cent, and 5 per cent thymol in chloroform. Toluene was, in general, the most satisfactory. Usually it was used to 2 per cent concentration, but where large surfaces were exposed, as high as 4 per cent was found necessary. The chloroform-thymol was also very efficacious, but in the carbohydrate experiments chloroform could not be used because of its power of reducing copper. In the lipase work the substrate was made up to 20 per cent alcohol since the action seemed to proceed best in the presence of this antiseptic. In all cases where the experiments were maintained over a considerable period of time, it was necessary to add additional antiseptic from time to time.

Checks were set up in all experiments—on the substrate, on the material used to demonstrate enzyme action, and on the substrate plus such enzyme material boiled to destroy any ferments that might be present.

CARBOHYDRASES OF THE ALGAE

In these experiments the alcohol precipitate from an aqueous extract of crushed, fresh or dried, algal tissue was employed as an enzyme source, this precipitate being diffused in such a volume of distilled water that one gram of the original material was represented by 5 cc. of the diffusion. Thus one can more closely compare the amounts of enzyme present in definite amounts of different algal tissue. The number of cubic centimeters of diffusion will be noted in connection with each set of experiments.

Substrates.—Starch, dextrin, inulin, sucrose, maltose, lactose, glycogen, and in one or two cases, laminarin isolated from *Laminaria Agardhii,* were used as substrates. These were made up in 1 per cent concentrations with the exceptions of maltose and glycogen, where .25 per cent, and laminarin, where .5 per cent concentrations were employed.

Of the many suggested methods for making up starch paste, the following one used by Clark ('11) was found to give the best satisfaction. Ten grams of potato starch were weighed out and placed in a beaker with 250–300 cc. of distilled water.

This was brought to a boil with constant stirring, and when an opalescent solution resulted the paste was transferred with rinsing to a 2-liter flask containing about 500 cc. of boiling water. The lot was boiled under a reflux condenser for two hours, cooled, and made up to a liter. Although, as is stated by Clark, this treatment is very effective in breaking down the starch grain physically, no detectable hydrolysis takes place, and the additional advantage is gained in obtaining a paste that will not settle out, even after long standing. Two per cent toluene was employed as an antiseptic if the starch were not to be used immediately.

Since all dextrin obtainable contained some reducing sugar, it was found necessary to purify it by making a concentrated solution in hot distilled water, and then precipitating out with several volumes of 95 per cent alcohol. The dextrin was caught on a filter paper and dried at a low constant temperature.

Laminarin, a dextrin-like carbohydrate found in many of the *Fucaceae,* was isolated from *Laminaria Agardhii* according to the method employed by Kylin ('13), with some few slight modifications. Freshly collected *Laminaria* was crushed in the usual way and 1,680 grams of the pulp were boiled with 7 liters of water for 24 hours, water being added from time to time to replace that lost through evaporation. The extract was then filtered off through a double thickness of cheese cloth, and the residue pressed out with a tourniquet. About 3,000 cc. of a dirty brown filtrate were obtained which was divided into three lots of 1,000 cc. each. To the first of these was added a concentrated $Ba(OH)_2$ solution until the precipitation of the inorganic matter was complete. The precipitate was caught on a cotton filter in a Buchner funnel, the filtrate being a clear, golden-colored liquid. The inorganic material in the other two lots was precipitated with basic lead acetate, the liquid filtered off through cotton, and the excess of lead removed with H_2S. The solutions were filtered while hot through double filter paper to remove the lead sulphide, and then the excess of H_2S was driven off with heat. The three portions were first evaporated to about one-

third their volume, when the scum that formed was filtered off; this filtrate was then further evaporated to about one-fifth the original volume on the water bath. At this point the two lead acetate portions were placed together. Ninety-five per cent alcohol was added to each of the lots to about 80 per cent concentration when a flocculent precipitate came down rather slowly. With the $Ba(OH)_2$ portion this was copious, with the lead acetate, slight. After two hours the precipitates were filtered off, washed with absolute alcohol, redissolved in a small amount of distilled water, and then reprecipitated with 4 volumes of absolute alcohol, the resulting precipitate being dried over $CaCl_2$. From the $Ba(OH)_2$ portion, 4.2 grams of a creamy white powder were obtained that gave a very slightly reddish tinge with iodine, did not reduce Fehling's, and was easily soluble in water, giving a clear solution. Upon hydrolysis with weak H_2SO_4 a reducing sugar was split off. The lead acetate portion gave but two grams of the same material. This powder was taken to be the laminarin described by Kylin.

The determination of reducing sugars.—The reduction of copper, or in the case of maltose and lactose, the increase in the reducing value of the substrate plus the enzyme over that of the checks, was taken as the measure of carbohydrate hydrolysis. In this determination the permanganate titration method, as modified and described by Shaffer ('14), was used, it being possible with it to determine amounts of sugars as low as 2 milligrams[1] very accurately and quickly. Shaffer's description may not be generally available to plant workers who may desire to use this really splendid method, and so the various steps in the process as used here are set down in some detail.

Ten cc. of the carbohydrate-enzyme substrate were placed in a large test-tube containing 5 cc. of water, and just brought to a boil. At this point a drop of 50 per cent acetic acid was added. When the slight protein precipitate formed, 5 cc. of

[1] Shaffer determines values below two milligrams, but as used here, consistent results could not be obtained where less than that amount was involved. Below this point the relative increase in the experimental error is large.

colloidal iron (Iron dialysed, Merck) were pipetted in and the tube well shaken, the iron then being flocked out with .25 gram of Na_2SO_4. Upon the addition of this latter the mixture was again thoroughly shaken and the iron precipitate thrown down by centrifuging, the resulting clear, supernatant liquid then being decanted off through a small filter. This filtrate was entirely free of proteins or other substances which, through oxidation later, would lead to errors in the permanganate values. Ten cc. of this filtrate were placed in a 50 cc. lipped centrifuge tube, and standard Fehling's solution added, the copper content of which was in excess of that reducible by the sugar present.[1] The tube was then placed in a boiling water bath for 10 minutes, at the end of which time it was centrifuged at a moderate speed for 2 minutes, the supernatant unreduced Fehling's carefully decanted off, a like volume of distilled water added, and the cuprous oxide again thrown down by a 2-minute centrifuging. All but 1 or 2 cc. of this wash water was carefully decanted off, and the copper dissolved in the smallest amount necessary of a mixture of equal parts of 10 per cent ammonium ferric sulphate and 50 per cent sulphuric acid. It was found that if the copper were stirred up with a glass rod just before dissolving, it went into solution more readily. The dissolved copper was titrated directly in the centrifuge tube against N/50 $KMnO_4$.[2]

By calculation it is found that 1 cc. of N/50 $KMnO_4$ is equivalent to 1.27 milligrams of copper, and for the conversion of this into glucose use was made of the table prepared by Shaffer.[3]

As stated by Shaffer, care must be observed on the three following points: (1) to eliminate all oxidizable substances other than sugar, (2) to titrate the cuprous oxide immediately after dissolving, (3) to use poor conductors of heat as containers of the centrifuge tubes in the water bath, else many broken tubes will result. As employed here, circular wire

[1] In the determinations made here this amount never exceeded 10 cc.

[2] It is necessary to titrate immediately after dissolving because of the danger of oxidation of the cuprous oxide. If larger amounts of sugar are concerned, N/10 $KMnO_4$ may be used.

baskets having wooden bottoms and tops were used, the tops containing holes large enough for the free insertion of the centrifuge tubes, and the bottoms, slight depressions into which the tubes might rest. It is always necessary to run blanks with Fehling's solution since some reduction always takes place. The cuprous oxide solvent must be free from ferrous iron, and this can be assured by the addition of a trace of permanganate.

Method of setting up experiments.—Fifty cc. of the substrate to be used were placed in 125 cc. Erlenmeyer flasks with 2 per cent toluene as an antiseptic. If the series were maintained longer than six weeks, another 2 per cent toluene was added. As previously noted, in these carbohydrate experiments the material used for enzyme action was an alcohol precipitate from a water extract of algal powder or pulp. This was diffused in water so that 10 cc. of the diffusion represented 2 grams of the original tissue. Usually this amount was added to the substrate to be tested. Duplicates and checks were set up in accordance with the following model series for starch:

1. 50 cc. starch, 10 cc. enzyme diffusion.
2. 50 cc. starch, 10 cc. enzyme diffusion.
3. 50 cc. starch, 10 cc. boiled enzyme diffusion.
4. 50 cc. starch, 10 cc. boiled enzyme diffusion.
5. 50 cc. starch, 10 cc. distilled water.
6. 50 cc. starch, 10 cc. distilled water.

[3] To make this table more generally available, it is printed here in full.

Shaffer's table of copper-glucose equivalents

mgms. copper	mgms. glucose	mgms. copper	mgms. glucose	mgms. copper	mgms. glucose
0.7	.47	6.0	2.74	20.0	9.71
1.0	.62	7.0	3.21	25.0	12.25
1.5	.88	8.0	3.68	30.0	14.80
2.0	1.11	9.0	4.15	35.0	17.40
2.5	1.32	10.0	4.65	40.0	20.00
3.0	1.50	12.0	5.61	50.0	25.00
3.5	1.67	14.0	6.61	60.0	30.10
4.0	1.82	16.0	7.61	80.0	40.40
5.0	2.27	18.0	8.65	100.0	50.70

In addition, at the end of a complete carbohydrate series there were included for each alga the following checks:

1. 50 cc. distilled water, 10 cc. enzyme diffusion.
2. 50 cc. distilled water, 10 cc. enzyme diffusion.
3. 50 cc. distilled water, 10 cc. boiled enzyme diffusion.
4. 50 cc. distilled water, 10 cc. boiled enzyme diffusion.

Where the enzyme diffusion referred to above actually contained carbohydrases, it was extremely difficult to render them inactive by heating—10 minutes at the boiling point not being sufficient in most cases to more than slow down the action. This was probably due to the impurities contained, the relatively large amounts of protein and slime present tending to protect the enzymes. Those extracts relatively richer in such constituents proved the more difficult to render inactive. The expedient was finally adopted of placing the enzyme material in the autoclave and bringing the pressure in the latter up to 15 pounds. This proved quite effective.

THE CARBOHYDRASES OF ULVA LACTUCA

The effect of an extract of Ulva lactuca on different starches.—Since starches were to be used in many of the following

TABLE II

THE ACTION OF ULVA LACTUCA "DIFFUSION-EXTRACT"* UPON CERTAIN STARCHES

Starch 50 cc. 1 per cent	15 days		30 days	
	Sugar as glucose in 5 cc.† mgms.	Iodine test	Sugar as glucose in 5 cc. mgms.	Iodine test
Potato	10.1	Blue, trace red	17.4	Complete hydrolysis
Arrowroot	9.8	Blue, trace red	16.9	Complete hydrolysis
Wheat	9.9	Blue, trace red	17.1	Complete hydrolysis
Corn	6.3	Blue	10.8	Reddish purple
Soluble	8.7	Blue	15.9	Traces dextrin
Inulin	....	.....	Trace‡	.....

* Wherever the term "diffusion-extract" is employed, it refers to a diffusion in water of the alcohol precipitate from an aqueous extract of the alga under discussion.

† The sugar values in this and the following tables are net, i. e., sugar values for all checks have been deducted.

‡ In all the following experiments an amount of sugar below 2 mgms. is designated a "trace."

experiments, it was desired to know which, if any, were the most favorable substrates for the diastases of the algae. The action of diastase from *Ulva lactuca* was taken as an index. Potato, arrowroot, wheat, corn, and soluble starch, as well as inulin, were made up in 1 per cent concentrations in the manner previously described. To 50 cc. of each of these substrates were added 10 cc. of a diffusion of an alcohol precipitate from a water extract of dehydrated *Ulva lactuca*. Two per cent toluene was added as an antiseptic, and the flasks maintained at a temperature of 35°C. for 30 days. The results of the experiments are given in table II.

The data show but slight differences in the rate of digestion of the starches with the exception of corn starch, and the reason for this is not clear. One would expect it to be due to some impurity in the starch rather than to an inherent difference in the granule. The action on inulin was so slight as not to warrant the assumption of hydrolysis due to inulase.

The action of an extract of Ulva lactuca upon various carbohydrates.—A series was arranged using a "diffusion-extract" from *Ulva lactuca* with the following substrates: potato starch, dextrin, glycogen, sucrose, maltose, and lactose. Ten cc. of the "diffusion-extract" were added to each flask with 50 cc. of substrate, 2 per cent toluene used as an antiseptic, and the flasks maintained at a temperature of 35°C. for 30 days. The data are given in table III.

TABLE III

THE ACTION OF AN EXTRACT OF ULVA LACTUCA UPON VARIOUS CARBOHYDRATES

Substrate	Sugar as glucose in 5 cc. mgms.	
	15 days	30 days
Starch	10.20	15.50
Dextrin	6.30	9.95
Glycogen	2.25	3.50
Sucrose	.....	Trace
Lactose	.....	.....
Maltose	.....	Trace

The two polysaccharides, starch and dextrin, are very readily attacked even though the action is slow. Glycogen, which is hydrolysed by most diastatic enzymes with about the

same ease as starch, seems very slightly acted upon by the carbohydrases of *Ulva.* The failure of action on sucrose and lactose is not so surprising as is that on maltose, for one would expect the action on polysaccharides to continue to what is generally held to be directly assimilable sugars, i. e., the hexoses.

THE CARBOHYDRASES OF ENTEROMORPHA INTESTINALIS

This series (table IV) was run under exactly the same conditions as the one preceding. The "diffusion-extract" was from dehydrated tissue about two months old. Ten cc. of this were used with each 50 cc. of substrate, toluene added as an antiseptic, and the flasks kept at a temperature of 35°C. for 30 days.

TABLE IV

THE ACTION OF A "DIFFUSION-EXTRACT" FROM AIR-DRIED ENTEROMORPHA TISSUE UPON CERTAIN CARBOHYDRATES

Substrate	Sugar as glucose in 5 cc. mgms.	
	15 days	30 days
Starch	9.7	13.1
Dextrin	5.1	9.8
Glycogen	2.8	3.9
Inulin	Trace	Trace
Sucrose	....	Trace
Lactose	....	....
Maltose	....	....

The results for this closely related form are consistent with those obtained for *Ulva,* the action in the present case, however, being somewhat slower. The more common polysaccharides are acted upon while the disaccharides are not attacked.

THE CARBOHYDRASES OF LAMINARIA AGARDHII

The water extract from air-dried Laminaria tissue was extremely viscous and upon addition of alcohol, a very heavy precipitate was thrown down that contained a large amount of algal slime. When water was added to this precipitate in the usual ratio a very viscous diffusion was obtained. Ten cc. of the "diffusion-extract" were used with 50 cc. of the substrate and 2 per cent toluene added as an antiseptic. The flasks were kept at a temperature of 20–22°C. for 100 days,

portions being removed and sugar determinations made at the definite intervals noted in table v.

The carbohydrases in this form appear to be limited to those acting on starch and dextrin, and with these the hydrolysis proceeds much more slowly than was true with either of the preceding "greens." The lower temperature at which the hydrolysis occurred does not explain completely the lessened action. Inhibiting substances or else an actually smaller concentration of the enzyme seem to be important factors.

TABLE V

THE ACTION OF A "DIFFUSION-EXTRACT" FROM AIR-DRIED LAMINARIA TISSUE UPON CERTAIN CARBOHYDRATES

Substrate	Sugar as glucose in 5 cc. mgms.			
	15 days	45 days	75 days	100 days
Starch	Trace	3.25	4.7	6.5
Dextrin	Trace	4.1	6.85	8.3
Glycogen	Trace	Trace	Trace	Trace
Inulin	....	....	Trace	Trace
Sucrose	....	....	Trace	Trace
Lactose	....	....	....	....
Maltose	....	....	Trace	Trace

Another series (table vi) of flasks was set up with the same form, using a "diffusion-extract" from the fresh tissue. Ten cc. of this diffusion represented 6 grams of the Laminaria pulp. In addition to the usual substrates, .5 per cent laminarin was used. Toluene was added and the flasks maintained for 60 days at room temperature (22–23°C.).

TABLE VI

THE ACTION OF A"DIFFUSION-EXTRACT" FROM FRESH LAMINARIA TISSUE UPON CERTAIN CARBOHYDRATES

Substrate 100 cc.	Sugar as glucose in 5 cc. mgms.				
	7 days	15 days	30 days	45 days	60 days
Starch	.....	Trace	2.7	4.2	5.35
Dextrin	Trace	Trace	3.6	5.15	7.40
Laminarin	Trace	2.4	3.9	5.4	5.65
Glycogen	Trace	Trace	Trace	Trace	Trace
Inulin	.....	.....	.....	Trace	Trace
Sucrose	.....	.....	Trace	Trace	Trace
Lactose	.....	.....	.....	.....	.....
Maltose	.....	.....	Trace	Trace	Trace

The diastases of fresh *Laminaria* seem slightly more active than those isolated from the dried tissue; however, no other carbohydrases were evident than those shown in the previous table.

THE CARBOHYDRASES OF ASCOPHYLLUM NODOSUM AND MESOGLOEA DIVARICATA

The Mesogloea material was dehydrated as soon as brought into the laboratory, the preliminary fresh-water washing being omitted because of the rapid gelatinization of the tissue. The crushed dried tissue, extracted in the usual way, gave a very heavy, stringy precipitate with alcohol, consisting, as did that from *Laminaria,* mostly of slime. This, when diffused in the usual volume of water, gave a very viscous mixture. Crushed fresh *Ascophyllum* was extracted directly. The viscosity of the extract was high, but the alcohol precipitate from it came down in a flocculent mass that gave only a slightly viscous diffusion with water.

Experiments were set up with the various carbohydrates heretofore employed, including laminarin, and in the different series, amounts of the "diffusion-extract" were used varying from 5–15 cc. As was true with the *Fucus* reported in the previous study, in no case were there evidences of hydrolysis even after 60 days at room temperature.

THE CARBOHYDRASES OF RHODYMENIA PALMATA

The air-dried Rhodymenia tissue proved to give rise to one of the most viscous extracts encountered in the algae, 20 volumes of water being necessary to make handling possible. With alcohol, a very rubbery, white precipitate came down that was made up of a large proportion of algal slime. This diffused very slowly, giving an extremely viscous mixture. Ten cc. of the "diffusion-extract" were used with the substrate to determine action, and toluene was added. The flasks were kept at a temperature of 21–22°C. for 100 days, sugar determinations being made from time to time, the results of which are given in table VII.

The results here are quite comparable to those obtained with *Ulva* and *Enteromorpha,* the same carbohydrates being

acted upon, although perhaps a little more slowly. This action is definitely progressive with starch, dextrin, and laminarin, but with glycogen it takes a sudden jump during the 15–45-day period, then remains practically stationary for the rest of the time the series is being maintained. As was true of the results shown in the previous tables, this carbohydrate was less favorable as a substrate than any of the other polysaccharides employed.

TABLE VII

THE ACTION OF A "DIFFUSION-EXTRACT" FROM AIR-DRIED RHODYMENIA TISSUE UPON VARIOUS CARBOHYDRATES

Substrate	Sugar as glucose in 5 cc. mgms.			
	15 days	45 days	75 days	100 days
Starch	9.2	12.2	14.8	18.2
Dextrin	8.25	9.7	10.3	11.1
Glycogen	Trace	6.1	6.4	6.7
Laminarin	4.7	7.3	9.6	10.5
Inulin	....	....	Trace	Trace
Sucrose	....	Trace	Trace	Trace
Lactose	....	....	....	Trace
Maltose	....	....	Trace	Trace

THE CARBOHYDRASES OF AGARDHIELLA TENERA

The very succulent nature of the freshly collected material compelled its partial dehydration immediately. Two 15-minute treatments with 95 per cent alcohol were used, then the tissue spread out on paper toweling to dry at room temperature. After drying, it was very easily powdered without the aid of quartz sand. The alcohol precipitate from a water extract of this powder was quite fine and flocculent, differing much from that of *Rhodymenia,* both in amount and in nature.

TABLE VIII

THE ACTION OF A "DIFFUSION-EXTRACT" FROM DEHYDRATED AGARDHIELLA TISSUE UPON CERTAIN CARBOHYDRATES

Substrate	Sugar as glucose in 5 cc. mgms.			
	15 days	45 days	75 days	100 days
Starch	6.35	9.4	14.5	20.9
Dextrin	7.00	10.5	15.95	19.7
Glycogen	2.35	6.15	6.65	6.85
Laminarin	5.8	8.3	11.6	13.2
Inulin	....	Trace	Trace	Trace
Sucrose	Trace	Trace	Trace	Trace
Lactose	....	....	....	Trace
Maltose	....	....	Trace	Trace

It diffused readily in water with no resulting viscosity. Ten cc. of the "diffusion-extract" were used for enzyme action, toluene added, and the flasks kept at a temperature of 21–23°C. for 100 days. The data here obtained are given in table VIII.

Dextrin here more nearly approaches starch as a favorable substrate, differing from the action evidenced by the other algae with the exception of *Laminaria,* where all action was slow. There is also a slightly increased action over that evidenced by *Rhodymenia,* for all the carbohydrates hydrolysed.

THE CARBOHYDRASES OF CERAMIUM RUBRUM

As was the case with *Rhodymenia,* it was necessary here to use 20 volumes of the water-extracting medium, not, however, because of the great viscosity, but on account of the great adsorption of water by the tissue particles. The alcohol precipitate was copious and finely flocculent. It diffused in water rather slowly, giving a mixture that was only slightly viscous. Ten cc. of the "diffusion-extract" were used for action, the usual percentage of toluene added, and the flasks maintained at a temperature of 21–23°C. for 100 days. The data are given in table IX.

TABLE IX

THE ACTION OF A "DIFFUSION-EXTRACT" FROM FRESH CERAMIUM TISSUE UPON CERTAIN CARBOHYDRATES

Substrate	Sugar as glucose in 5 cc. mgms.			
	15 days	45 days	75 days	100 days
Starch	6.85	8.1	11.75	16.9
Dextrin	11.5	15.0	17.5	19.6
Glycogen	Trace	6.2	7.3	8.85
Laminarin	7.2	9.4	12.1	12.2
Inulin	....	....	Trace	Trace
Sucrose	....	Trace	Trace	Trace
Lactose	....	....	....	Trace
Maltose	....	Trace	Trace	Trace

Dextrin proved the most favorable substrate for the carbohydrate enzymes of this alga, the hydrolysis being about the same as that evidenced by *Agardhiella.* With the exception of glycogen, the other carbohydrates showed a decreased hydrolysis when compared with this latter form, and when

compared with *Ulva* the difference is quite marked. As in the other algae, *Ceramium* showed no ability to hydrolyse the disaccharides used.

A COMPARISON OF THE DIASTATIC ACTIVITY OF ULVA LACTUCA WITH THAT OF LEAF TISSUE FROM SOLANUM TUBEROSUM

One of the very evident facts brought out by the data in the preceding tables was the relative slowness with which hydrolysis was carried on. This point made it seem worth while to compare, in a general way, the activity of such a form as *Ulva* with the starch-forming leaf tissue of a higher plant, one from which diastase could be isolated rather easily. The potato (*Solanum tuberosum*) was chosen.

The Ulva tissue was from an air-dried lot that had been tried out earlier and had been found quite active. Fresh potato tops were brought into the laboratory, and both these and the *Ulva* given the "dauerhefe" treatment. After dehydrating and drying at room temperature, both lots were ground in a mill, then reduced to a fine powder in a mortar. Exactly 18.5 grams of each were extracted with 250 cc. of water for 12 hours at room temperature with toluene added as an antiseptic, and then the protein-enzyme complex precipitated with 2.5 volumes of 95 per cent alcohol. The Ulva precipitate was the characteristic heavy white mass to which attention has been called before, while that of the potato was finely divided and dark.

The entire amount of each precipitate was diffused in 60 cc. of water. The Ulva precipitate gave a rather viscous diffusion, due to the adsorption of water by the protein particles; that from the potato did not all go into solution, making it necessary to shake the flask so that a true sample might be obtained. Five cc. of the "diffusion-extract" represented 1.84 grams of the original dehydrated tissue, and this volume was used with 50 cc. of a starch and dextrin substrate. Toluene was added as an antiseptic, and the flasks kept at a temperature of 31°C. for 42 days. Portions of the substrate were removed from time to time and sugar determinations made, the results of which are shown in table x.

The action of the potato extract upon starch was about two and one-half times that of *Ulva,* and its action on dextrin about twice in all of the determinations made. For some unknown reason the hydrolysis of dextrin by the diastase from *Ulva* ceased after the twenty-eighth day.

TABLE X

A COMPARISON OF THE DIASTATIC ACTIVITY OF ULVA WITH THAT OF POTATO LEAF TISSUE

Substrate 50 cc.	Sugar as glucose in 5 cc. mgms.									
	14 days		21 days		28 days		35 days		42 days	
	Ulva	Potato	Ulva	Potato	Ulva	Potato	Ulva	Potato	Ulva	Potato
Starch....	8.7	18.1	9.6	26.3	11.8	28.1	12.9	33.5	13.8	35.5
Dextrin...	10.5	17.3	11.5	25.1	17.8	27.5	17.9	30.3	17.9	31.9

ACTION OF VARIOUS ALGAL EXTRACTS UPON THE CARBOHYDRATE CONSTITUENTS OF AGAR-AGAR, AND OF VARIOUS GUMS, AS WELL AS EXPERIMENTS UPON THE AUTOLYSIS OF ALGAL SLIME

Because of the large amounts of carbohydrate-containing slime formed by many algae, and because of the rôle this might play as a reserve product, it was deemed advisable to try out the various algae for enzymes capable of hydrolysing such complex carbohydrates to assimilable sugars. It was assumed on the basis of the work done by König and Bettels ('05) and others, that such hydrolytic products would be reducing sugars, in all probability galactoses and pentoses.

A series was set up with each of the several algae, using 50 cc. of .25 per cent agar as a substrate and varying amounts of a "diffusion-extract" from fresh tissue. The agar substrate was slightly viscous in the cold, but when kept at a temperature of 40°C., the optimum temperature for diastase, this was not noticeable. Toluene was used as an antiseptic. The flasks were shaken at regular intervals during a 30-day period and at the end of that time aliquot portions were removed and tested for reducing sugars. There was no reduction in any case.

As a parallel series, thin strips of agar were placed in test-tubes and 20 cc. of "diffusion-extract" added. Toluene was used as an antiseptic and the tubes kept at a temperature of 40°C. for two months. At the end of that time no hydrolytic

action was observable, either by reduction of Fehling's or by microscopical examination.

In the experiments on the hydrolysis of various poly- and disaccharides, checks were set up in which the usual amount of "diffusion-extract" was placed in distilled water. This was to determine the reduction of copper, if any, due to the "diffusion-extract" itself. In no case was there more than a very slight trace that might have been due to other causes than enzymic. However, it was thought that a self-digestion series would more definitely determine whether the hydrolysis of the carbohydrates of the slime could be brought about by specific algal enzymes. With this in mind, a series was arranged in which the flasks contained 50 cc. of a water extract from each of the forms investigated. Checks were set up in which the "diffusion-extract" was inactivated in the autoclave. Toluene was used as an antiseptic and the flasks maintained at a temperature of 22–23°C. for two months. Aliquot portions removed from time to time failed to show the slightest trace of hydrolysis.

It will be remembered that Tihomirov ('10) had found osozone-forming sugars in the conceptacles of *Ascophyllum* and *Fucus* that he thought might be dextrose and d-galactose, possibly also fucose and arabinose. Thinking that these might possibly have arisen from their corresponding anhydrides contained in the conceptacle slime, a self-digestion series was set up with an extract from the abscised, crushed conceptacles of those two forms. The *Fucus* was in a fruiting state. The series were set up in duplicate, one kept at room temperature and the other at 32–33°C. Fehling's test showed no hydrolysis after a month.

Pentosans alone were then used as substrates. Two series of flasks for each of the algae investigated were set up, each containing a .5 per cent solution of gum arabic.[1] To one series was added 10 cc., to the other 20 cc. of "diffusion-extract," and the flasks placed at room temperature with toluene as an antiseptic. No hydrolysis was apparent either

[1] The gum arabic was dissolved in water, then precipitated with several volumes of 95 per cent alcohol to get rid of reducing sugars.

by the phloroglucin test or by sugar determinations, even after 60 days.

THE ACTION OF ALGAL "DIFFUSION-EXTRACTS" UPON CELLULOSE AND HEMICELLULOSE

Experiments were carried out to determine the presence or absence of cellulose hydrolysing enzymes in the algae, and to this end several methods were employed. First, strips of filter paper were placed in test-tubes and entirely covered with 20 cc. of "diffusion-extract." Checks were maintained with distilled water and also with the "diffusion-extract" alone. The series were set up in duplicate—one kept at room temperature and the other at 35°C., both with toluene as an antiseptic. After definite intervals during a 60-day period, the contents of the tubes were tested for reduction. None was observable in any case, and microscopic examination of the filter paper failed to reveal any decomposition whatsoever.

A double series was then set up in a similar way, except that 2 grams of fresh, crushed algal tissue were added to the tubes instead of the "diffusion-extract," together with 20 cc. of distilled water. At the periods noted above, microscopic examination revealed no attack. It was thought an inherent difference between algal and filter paper cellulose might be responsible for this absence of action. Accordingly, cellulose was prepared from the tissue of *Ascophyllum* after the method described by Fowler ('11, p. 159) and used by Cooley ('14). Fifty grams of air-dried tissue were placed in a liter flask, 500 cc. of distilled water added, and the lot placed in the autoclave at 15 pounds for 15 minutes to destroy any cellulase that might be present, and also to extract as much as possible of the water-soluble substances. The water was filtered from the tissue, fresh water added, and the flask placed in an incubator at 35°C. It was kept at this temperature with daily changes of water for 10 days, at which time the water-soluble constituents seemed to be almost entirely removed. The treatment from here on was the same as that described by Cooley. To the tissue was added a liter of potassium-chlorate-nitric-acid solution made up in the proportion of 30 grams of potassium chlorate to 520 cc. of nitric

acid (sp. gr. 1.1). The flask was kept in the ice-box for two weeks, when the oxidizing mixture was changed and the new lot allowed to remain another fortnight. At the end of this time a yellowish white tissue was obtained, representing fairly pure algal cellulose. This was filtered off, washed well with distilled water, and dried in the oven at 75–80°C. The final product weighed 19.7 grams.

This cellulose was used in a way similar to the filter paper in the first series. One gram was placed in each flask and well shaken up with 50 cc. of distilled water. A concentrated "diffusion-extract" was prepared from *Ascophyllum, Laminaria, Ulva,* and *Chondrus,* 10 cc. of which represented 5 grams of the original dried tissue. This volume was added to the flasks, and the series set away at 30°C. with toluene as an antiseptic. At the end of two months no reduction of Fehling's was observable and under the microscope there seemed to be no decomposition of the cellulose particles.

Action on hemicelluloses.—Hemicellulose was used from two sources—from date seeds, and from the seeds of the wild persimmon, *Diospyros virginiana.* In both cases the experiments were essentially the same. The horny coats were broken and the embryos removed. Small pieces of the hemicellulose were then taken, placed in a flask with water, and heated in the autoclave at 15 pounds for 15 minutes to kill the cytase present. Upon removal from the autoclave the pieces were washed several times in distilled water, being left in the last wash water for several days with toluene as an antiseptic—this to get rid of any reducing sugars present. Two of these washed pieces were placed in test-tubes with 10 cc. of the concentrated "diffusion-extract" used in the experiments with cellulose. Another lot was covered with 10 cc. of distilled water and 2 grams of the dried algal powder added. In a third series shavings of the hemicelluloses were mounted in a Van Tieghem cell with a drop of enzyme solution. All the algae under investigation were tried out, but in no case was there the slightest trace of decomposition, either microscopically or by the reduction of copper.

Results.—These negative results do not necessarily argue against the production of slime through the agency of enzymes. It is impossible to exactly reproduce the conditions of the cell *in vitro,* and enzymes which might act upon cellulose in the living tissue to produce slime might easily be inhibited from action on cellulose or hemicellulose under the conditions of the experiments. Grüss ('10) found that fresh cherry gum contained cytase, but that none was demonstrable in the older gum. He also found that malt diastase would not act upon such gum until the tannins had been removed. It is known that the algae do contain tannins or "tannoidal" bodies, the writer having demonstrated a "tannoid" content in *Ascophyllum* of 1.1 per cent of the dry weight. These, or other agents, could be involved in the partial or complete inhibition of cytolytic action. On the other hand, indirect evidence, at least, points to the presence of the galactan and pentosan groups as due to their being laid down as such, that is, they do not arise as the direct result of hydrolytic enzyme action, but probably represent the final step in the condensation of those particular hemicelluloses. Tschirsch ('89) and his students have shown that the algal slime exists as an intracellular substance, and they hold that in most instances, at least, it does not arise from the cellulose. This seems to be the logical view, and we in turn seem justified in looking upon the galactan and pentosan groups in the algae as normal products of the plant's metabolism, present at all stages in the plant's growth, and capable of giving rise to gelatinization at any time upon the adsorption of water. If one examines, for instance, such forms as *Fucus, Mesogloea,* and *Chondrus,* the slime is hardly detectable when the plants are growing under normal conditions, but when brought into the laboratory and placed in fresh water, a rapid adsorption begins at once. The dissolved salts in sea-water are undoubtedly the inhibiting factors in such adsorption under normal conditions.

That this inhibition is not bound up with the living cell may be shown by the simple experiment of killing two fronds of *Chondrus,* for example, and placing one in fresh, the other

in salt water, with toluene to keep down bacterial action. Very slight, if indeed any, gelatinization is evident with the frond placed in salt water, while that in fresh water begins to gelatinize immediately. It is also a well-known fact that in histological or cytological work with these forms, the killing fluids must be made up in sea-water or water containing a high percentage of salts, else gelatinization interferes. These facts, together with the apparent absence of cellulase and cytase, tend to show that the galactan and pentosan groups are always present as final condensation forms of their particular "generic" carbohydrate line, and that sliming in the marine algae, at least, is the result of the adsorption of water by these already existing carbohydrate groups.

DISCUSSION OF RESULTS OF CARBOHYDRASE EXPERIMENTS

It is seen from the data presented in the foregoing tables that carbohydrases in the algae, at least those that can be isolated by standard methods, are very few. Furthermore, in all cases where such carbohydrase action is evident, it is limited to the polysaccharides—starch, dextrin, laminarin, and glycogen. In no case were the disaccharides hydrolysed. As groups, the "greens" are more active than the "reds," while of the "browns," *Laminaria* is the only form in which carbohydrate action is demonstrable. Moreover, the action here is extremely slow and is limited to starch, dextrin, and laminarin. *Mesogloea* and *Ascophyllum* are similar to *Fucus* in failing to show the presence of carbohydrases. Within the groups there is little difference in the rate of carbohydrase action. This is especially true in the "greens." Of the "reds," *Agardhiella* is a little more active than the other forms investigated, while *Ceramium* is slightly the slowest. Bartholemew ('14), in the work already referred to, also found that *Ceramium* was less active than the other "reds" with which he worked.

The various polysaccharides, with two exceptions, prove favorable as substrates for the various algae in the same order, viz., starch, dextrin, laminarin, and glycogen. The carbohydrases of *Ceramium* act more rapidly upon dextrin than upon starch and this is also true of *Laminaria,* although

to a lesser extent. Glycogen, which is very generally hydrolysed by diastase, is here decidedly less readily attacked than the other polysaccharides. This would seem to indicate that we are dealing with a distinct enzyme, one that might be placed in the same category with dextrinases. These latter always occur with the diastases but are held by many workers to be distinct.

Some of the substrates tested for hydrolysis do not, as far as we know, occur in the plants investigated. This is true of sucrose, lactose, and inulin. However, although this might reconcile us to the failure to find their specific enzymes, it does not argue conclusively against such enzymes being formed. It is well known that tissues do form ferments that have no detectable substrates upon which to act—the rennen of the bird's stomach and the urease of the Soja bean being notable examples. Inulin, as pointed out previously, does occur in certain "greens," as in *Acetabularia* and members of the *Dasycladaceae.* Unfortunately, none of these forms were available for investigation.

The absence of lactase and sucrase is not so significant as is that of maltase. It is very generally considered that in the plant, as well as in the animal organism, poly- and disaccharides must be hydrolysed to simple sugars before assimilation can take place. It is hardly possible that the algae are an exception to this general rule and yet it is difficult to account for this important negative result. It is known that inhibiting agents do not affect all enzymes alike, and it may be here that if such agents are liberated on the death of the cell, the maltase might prove more sensitive to them than the other carbohydrate enzymes. According to the findings of Kylin ('13), both dextrose and fructose have been demonstrated in the tissues of *Ascophyllum, Fucus,* and *Laminaria,* but in extremely small quantities. These results would tend to convince one that an enzyme giving rise to them is probably present in the algal cell.

Such carbohydrates as galactans, pentosans, and mannans, are very frequently met with in the algae and are potentially capable of being split to assimilable sugars. That they are

not so split, however, seems evident, at least not through the activity of demonstrable algal enzymes, and in the face of the negative evidence obtained, we would consider them as by-products of metabolism rather than as playing the rôle of reserves. As such, they would not be so comparable to the reserve carbohydrates of the date as they would be perhaps to the mucilaginous constituents of various seeds, as those of flax, mistletoe, etc. These latter adsorb water readily with gelatinization, and as far as is known, never function as reserves but act in a purely mechanical way (Czapek, '13, p. 705).

LIPASES IN THE ALGAE

The almost universal presence of fats in the marine algae led to the question of their assimilation. Accordingly, experiments were set up to determine the lipolytic activity upon emulsions of neutral fats as well as upon certain esters of the lower fatty acids. For the neutral fats olive oil was chosen as a substrate, and two general methods were employed in forming the emulsion.

The first, an olive oil-casein emulsion was made up after a method described by Bloor ('14). Four grams of casein were placed in a warm mortar on a water bath and water added until the whole formed a paste of medium viscosity. A drop of phenylphthalein was added, then N/1 NaOH poured in and stirred with the casein until the latter had been dissolved, this point being indicated by a permanent pink tinge of the mixture. Eight cc. of olive oil were stirred into the hot solution and then ground with a pestle until all the oil globules had disappeared. At this point the mortar was removed from the bath and the emulsion cooled. During the cooling it was found necessary to stir the mixture occasionally. The thick, creamy mass resulting was diluted up to the required concentration by the careful addition of water. If this dilution is too great, the oil globules tend to rise to the surface.

The second method was also suggested by Doctor Bloor, but, as far as is known, has not been described. Eight cc. of olive oil were dissolved in the smallest amount of absolute alcohol necessary. This solution was run through a hot fun-

nel to which a drawn-out piece of glass tubing had been attached, into about 100 cc. of cold distilled water, the water being stirred constantly while the olive oil was being run in. A milk-white emulsion made up of extremely small suspended globules of oil resulted. In an emulsion carefully made, most of these globules are small enough to show Brownian movement. The alcohol was driven off finally by heating and the emulsion made up to the desired concentration.

Both emulsions stand up well. In the latter, however, there is a tendency toward flocking out by some of the smaller particles upon the addition of any salt-containing substance, such as, for instance, algal powder; but, on the other hand, it has the advantage of being more easily checked up because of its simpler composition.

TABLE XI

LIPOLYTIC ACTION OF THE SEVERAL ALGAE UPON OLIVE OIL-CASEIN EMULSION

Alga	Number cc. of N/10 NaOH to neutralize 10 cc. of substrate											
	4 days				10 days				15 days			
	Emulsion + tissue	Emulsion	Water + tissue	Net acidity	Emulsion + tissue	Emulsion	Water + tissue	Net acidity	Emulsion + tissue	Emulsion	Water + tissue	Net acidity
Ulva	....	....	....	....	1.3	.25	.1	.95	....	....	.....	.....
Enteromorpha	....	....	....	....	1.25	.2	.1	.95	....	....	.....	.....
Mesogloea	.6	.00	.1	.5	1.00	.1	.1	.8	2.2	.6	.075	1.525
Ascophyllum	....	....	....	....	1.50	.05	1.0	.45	....	....	.....	.....
Laminaria	....	....	....	....	.4	.00	.05	.35	.6	.1	.025	.475
Chondrus	1.2	.00	.05	1.15	1.85	.00	.05	1.8	2.3	....	.1	2.200
Agardhiella	....	....	....	....	.3	.00	.05	.25	....	....	.....	.....
Ceramium	....	....	....	....	1.6	.00	.1	1.5	....	....	.....	.....
Rhodymenia	.3	.1	.05	.15	.9	.2	.15	.55	....	....	.....	.....
Champia	.2	.1	.05	.05	.35	.3	.05	.00	....	....	.....	.....

In all the lipolytic experiments, algal powder or fresh algal tissue crushed with fine quartz sand, was used as a source of enzyme action. In some of the original series the olive oil-casein emulsion was employed, but on account of the danger arising from a possible hydrolysis of the casein with a resulting increase in acidity, the alcohol emulsion was used in the later work.

Lipolytic action of the several algae upon olive oil-casein emulsion.—In this experimental series (table xi) flasks were set up containing 50 cc. of olive oil-casein emulsion as a sub-

strate, 5 grams of crushed algal tissue for enzyme action, and 10 cc. of 95 per cent alcohol as an antiseptic. Checks were employed wherein the flasks in one case contained the emulsion alone, and in another case, the same weight of algal pulp in distilled water. The flasks were maintained for 15 days except for the forms especially noted. At intervals 10 cc. portions were removed and titrated against N/10 NaOH with phenylphthalein as an indicator.

Lipolytic action on alcohol-water-olive oil emulsion.—Because of the possibility of the hydrolysis of the casein in the emulsion used in the preceding experiments, a series (table XII) employing the alcohol-water emulsion was set up as a check. This emulsion alone was practically neutral but a

TABLE XII
LIPOLYTIC ACTION OF THE SEVERAL ALGAE UPON ALCOHOL-WATER-OLIVE-OIL EMULSION

Alga	Number cc. N/10 NaOH to neutralize 10 cc. substrate after 10 days				
	Emulsion + tissue	Emulsion alone	Water + boiled tissue	Water + tissue	Net acidity
Ulva	.9	.00	.05	.05	.85
Enteromorpha	.8	.00	.025	.1	.7
Mesogloea	.65	.00	.05	.075	.575
Ascophyllum	.2	.00	.15	.15	.05
Laminaria	1.15	.00	.02	.3	.85
Chondrus	1.45	.00	.1	.35	1.10
Agardhiella	.25	.00	.05	.05	.20
Ceramium	.85	.00	.1	.20	.65
Rhodymenia	.525	.00	.05	.15	.375
Champia	.125	.00	.15	.15	.00

slight acidity was produced by the addition of the algal powder. A negligible amount of the oil globules ran together and collected at the surface of the liquid after some days, but the bulk of the emulsion stood up well. As in the preceding series, 5 grams of the fresh tissue were used as a source of lipolytic activity, and the alcohol in which the olive oil had been dissolved served as an antiseptic. Fifty cc. of the emulsion were used as a substrate, and the flasks maintained at a temperature of 22–23°C. for 10 days.

Lipolytic action on triacetin.—The lipolytic activity of dry tissue powder of *Ulva, Mesogloea,* and *Chondrus* was tested, using a .5 per cent solution of triacetin as a substrate. Two

grams of the tissue powder were used, otherwise the series (table xiii) was arranged exactly as the preceding and kept at room temperature for 25 days.

Action on other esters.—A series was set up with methyl acetate, ethyl acetate, and ethyl butyrate in .25 per cent solution, using 2 grams of algal powder with 50 cc. of the sub-

TABLE XIII

THE ACTION OF POWDERED TISSUE FROM CERTAIN ALGAE UPON TRIACETIN

Alga	Number cc. of N/10 NaOH to neutralize 10 cc. substrate after							
	10 days				25 days			
	Tri-acetin +tissue	Water+ tissue	Tri-acetin alone	Net acidity	Tri-acetin+ tissue	Water+ tissue	Tri-acetin alone	Net acidity
Ulva.....	.3	.025	.1	.175	.5	.15	.15	.2
Mesogloea.....	.25	.05	.1	.1	.4	.2	.15	.05
Chondrus.....	.55	.35	.1	.1	.8	.4	.15	.25

strate in 20 per cent alcohol. Titrations were made from time to time against N/10 NaOH with phenylphthalein as an indicator. Even after 60 days at room temperature no increase in acidity was observable over the checks.

General results for experiments with lipases.—The results serve to show that, although slight, there is distinct lipolytic activity in most of the forms investigated. The various groups of algae are not so distinct regarding this activity as was the case with the carbohydrases, nor does the activity of the individual alga in this case relate itself particularly to the activity shown by the form in its carbohydrase action. *Agardhiella* hydrolyses the polysaccharides more rapidly than any other alga, yet its lipolytic activity is very low. Likewise, *Laminaria,* so inactive in the previous group of enzymes, is among the most active on fats. *Fucus,* on the other hand, was found in previous work to have no action on either carbohydrates or fats.

The action is especially evidenced by use of the olive oil-casein emulsion. In general, the increases were less where the alcohol-water emulsion was used—a difference probably ex-

plainable on the ground that the casein gave rise to a slight acidity.

Specificity of action might explain the failure to obtain action on most of the esters. Euler ('12) differentiates the lipases into true lipases and esterases, the former acting on neutral fats particularly, the latter on the methyl and ethyl esters of the lower fatty acids. Even in this latter restricted field, great specificity may be shown. Reed ('12) found that ethyl acetate was quite rapidly acted upon by an esterase isolated from *Glomerella rufomaculans,* while ethyl butyrate was only slightly hydrolysed.

THE PROTEINASES

The proteolytic activity of the various algae was tested on albumin, casein, legumin, peptone, gelatin, and in certain cases, on proteins isolated from the algal tissue—most of these under acid, alkaline, and neutral conditions. The first four were made up in 1 per cent concentrations. Albumin and peptone went into solution quite readily; legumin and casein, being insoluble in water, were either weighed out directly, or dissolved in N/10 NaOH. The albumin and gelatin were also tested in the form of Mett's tubes, and the gelatin alone in test-tubes where it was held at a temperature high enough to keep it in a liquid state while in contact with the algal powder. In all cases, algal tissue was used directly, either fresh crushed, or dry powdered—usually 2 grams of the powder or 5 grams of the fresh tissue to each 50 cc. of substrate.

Determination of hydrolysis.—Proteolytic action was determined in several ways, each acting as a check on the others. The biuret test was used for the demonstration of tryptic action, the proteins being precipitated by $(NH_4)_2SO_4$ in saturated solution and the test applied in the usual way. The tryptophane test was employed for ereptic action and this also furnished a check on the action of trypsin. In this, 1 cc. of the protein solution was placed in a small evaporating dish, a drop of glacial acetic acid added, and then a few drops of strong chlorine water. The hydrolysis to the amino acid stage

was also demonstrated in two other ways—by the formaldehyde-titration method of Sörenson ('08), and the determination of the amino-nitrogen by the micro-Kjeldahl method of Folin ('13). The Sörenson method consisted in adding 2 cc. of formalin, made alkaline to a faint pink tinge with N/20 NaOH, to 10 cc. of the filtered protein solution, made alkaline to the same color. Upon mixing, the color disappeared and the acidity resulting was titrated against N/50 NaOH, using phenylphthalein as an indicator.

In the determination of the amino-nitrogen by the "micro" method of Folin, the protein in a 5 cc. filtered portion of the solution was precipitated with 2 cc. of a 25 per cent solution of phosphotungstic acid in 5 per cent H_2SO_4. The precipitate was filtered off and a 2 cc. portion of the filtrate removed for the determination of the nitrogen. Duplicate determinations were made in all cases. These portions were placed in Jena test-tubes, 20×200 mm., 1 cc. concentrated H_2SO_4 added, then 1 gram of K_2SO_4, and a drop of 5 per cent $CuSO_4$. The digestion was carried on over the flame from a micro burner, the fumes being carried away by the fume adsorbers described by Folin. Usually 20 minutes sufficed for the completion of the digestion, although in a few instances 25 minutes were required. After cooling slightly, 6 cc. of distilled water were carefully added. The tubes were then transferred to the distilling apparatus where concentrated NaOH was added to alkalinity, and the tube contents distilled over for three minutes, the NH_3 being collected in a known volume of N/10 HCl. The acid in the collection flask was titrated against N/10 NaOH with alizarin red (alizarin sulfonsäure Natrium, Merck), and the amount of nitrogen represented by the acid neutralized, determined.

In the method originally described by Folin, the NH_3 was not distilled but was forced over from an alkaline solution by a strong air current. However, students in his laboratory have made use of a micro distilling apparatus, and the suggestion for the ones employed here owes its origin to one of Folin's assistants. Distillation has the advantage of quickness, and from the writer's experience, of accuracy as well,

at least where suction instead of compressed air is employed in the air method. The results with the air current were very often below the theoretical. The distilling tubes used were made in the laboratory from glass tubing, the outer jacket measuring 40×2 cm., and the inner being 5 mm. in diameter. The lower end of this latter, where it dipped into the collection acid, was fitted with a larger tube 14 mm. in diameter—this to prevent a back flow of the acid; to the upper end of this inner tube was attached a safety bulb made from a 10 cc. pipette, and this in turn fitted into the Jena tube containing the distilling mixture, by means of a two-hole rubber stopper. Through the second hole in this stopper was a small piece of glass tubing closed at the upper end with a bit of rubber tubing and a pinch clamp; it was through this that the alkali was added after the apparatus was connected up for distillation.

Considerable trouble was experienced at first with bumping, especially after the digestion mixture had become concentrated. Neither bits of glass nor pebbles would overcome it. Finally the expedient was adopted of using short pieces of glass tubing sealed at one end and this end placed uppermost. These were of such a diameter that after digestion, the digestion mixture drawn up into them by the cooling of the contained air, would easily drain out when the boiling tube was forced up on the side of the test-tube by a quick downward motion.

The action of Enteromorpha, Mesogloea, and Chondrus powder upon various proteins.—Fifty cc. lots of casein, legumin, albumin, and peptone were used as substrates in this series—all in 1 per cent concentrations. The albumin and peptone were dissolved directly in distilled water, the legumin and casein in N/10 NaOH. Two grams of air-dried tissue powder were used for proteolytic action, with the exception, however, of *Mesogloea,* which, as before stated, was partially dehydrated before being air-dried. The various substrates were made neutral by the addition of N/10 alkali and then acid or alkaline by further addition of 2.5 cc. of N/10 HCl or NaOH. In the formaldehyde titrations 10 cc. of the sub-

TABLE XIV

THE ACTION OF TISSUE POWDER FROM CERTAIN ALGAE UPON VARIOUS PROTEINS

Substrate 50 cc.	Weight algal powder gms.	Reaction* of substrate	*Enteromorpha*				*Mesogloea*				*Chondrus*			
			Formol titration †		Biuret	Trypto-phane	Formol titrations		Biuret	Trypto-phane	Formol titrations		Biuret	Trypto-phane
			15 days	30 days			15 days	30 days			15 days	30 days		
Albumin	2	neut.	2.20	3.70	+	+	1.40	2.30	+	+	2.70	5.10	+	+
	.....	neut.	.30	.40	—	—	.50	.70	—	—	.40	.65	—	—
	2	acid	.80	1.90	+	—	.70	.90	—	—	1.65	2.85	—	—
	.....	acid	.40	.55	—	—	.40	.40	—	—	.65	.80	—	—
	2	alk.	2.75	4.95	+	+	2.15	4.80	+	+	2.10	4.35	+	+
	.....	alk.	.25	.30	—	—	.45	.55	—	—	.75	.75	—	—
Peptone	2	neut.	3.25	7.10	.....	+	2.70	5.75	.....	+	4.95	12.05	.....	+
	.....	neut.	.90	1.10	.....	—	.75	.80	.....	—	.90	1.05	.....	—
	2	acid	1.35	4.75	.....	+	1.50	4.35	.....	+	1.75	3.80	.....	+
	.....	acid	1.15	2.05	.....	—	.80	.95	.....	—	.60	.60	.....	—
	2	alk.	4.50	9.30	.....	+	3.55	7.90	.....	+	5.30	12.55	.....	+
	.....	alk.	.95	1.20	.....	—	1.10	1.10	.....	—	.85	1.20	.....	—
Casein	2	neut.	3.175	11.80	+	+	2.30	2.45	—	—	3.10	7.15	+	+
	.....	neut.	2.40	2.70	—	—	2.50	2.55	—	—	2.20	2.25	—	—
	2	acid	2.75	3.40	—	—	2.10	2.35	—	—	2.75	2.65	—	—
	.....	acid	2.60	2.70	—	—	2.40	2.45	—	—	2.80	2.95	—	—
	2	alk.	4.65	12.30	+	+	2.60	2.75	—	—	5.30	10.15	+	+
	.....	alk.	2.10	2.45	—	—	2.30	2.20	—	—	2.10	2.35	—	—
Legumin	2	neut.	2.10	4.95	+	+	1.55	1.70	—	—	2.10	2.45	—	—
	.....	neut.	1.80	1.95	—	—	1.90	2.00	—	—	1.85	1.65	—	—
	2	acid	2.00	3.60	+	+	1.40	1.65	—	—	1.70	2.30	—	—
	.....	acid	1.60	1.65	—	—	1.65	1.75	—	—	1.55	1.60	—	—
	2	alk.	3.35	7.10	+	+	1.95	2.15	—	—	2.90	5.85	+	+
	.....	alk.	2.10	2.05	—	—	1.50	1.90	—	—	.40	.80	—	—
Water	2	.....	.20	.25	—	—	.35	.30	—	—	.65	.75	—	—

*The substrates were made N/200 acid or alkaline with N/10 HCl or NaOH.
†The values represent the cc. of N/50 NaOH to neutralize 10 cc. of the substrate used.

strate were titrated against N/50 NaOH. One per cent chloroform-thymol was used as an antiseptic, and the flasks were kept at a temperature of 22–23°C. for 30 days.

The forms used in table XIV show a general ability to hydrolyse proteins. All four proteins employed were acted upon by one alga or another, but peptone and casein in neutral and alkaline solution were the most readily attacked. *Enteromorpha* split albumin and legumin but poorly; *Chondrus* acted upon legumin only in alkaline solution, and then slightly; *Mesogloea* failed to hydrolyse casein and legumin, and its action on albumin and peptone was very slow.

The action of Ulva, Laminaria, and Agardhiella powder on peptone and casein in alkaline and neutral solution.—Indications in the preceding experiment seemed to point to the fact that peptone and casein were more easily acted upon than the other proteins—and these more especially in neutral and alkaline solution. Accordingly, a series was set up with these two substrates, similar in all respects to the preceding one, except that the acid substrate was omitted and that *Ulva, Laminaria,* and *Agardhiella* were used for proteolytic action. Five grams of air-dried tissue were employed with 100 cc. of substrate. One per cent chloroform-thymol served as an antiseptic, and the flasks were kept at 35°C. for 30 days. Formaldehyde titrations were made after 15 and 30 days and tryptophane tests and amino-nitrogen determinations after 30 days. In the titrations 10 cc. of substrate were titrated against N/50 NaOH, and the amino-nitrogen represents that in 2 cc. of the filtrate from phosphotungstic precipitated protein.

The data in table XV tend to substantiate that of table XIV concerning the hydrolysis of peptone and casein. The higher temperature at which the flasks were maintained undoubtedly had something to do with the larger amounts of amino acids split off from these two proteins than was the case in the preceding series, yet if we can judge by the action on carbohydrates and fats, we are dealing here with the more active members, enzymatically, of their respective groups.

On the whole, peptone and casein seem to be the most favorable substrates of those used for proteolytic activity, and

TABLE XV

THE ACTION OF POWDERED TISSUE FROM CERTAIN ALGAE UPON PEPTONE AND CASEIN

Substrates 100 cc. 1 per cent	Weight algal powder gms.	Reaction of substrate	*Agardhiella*				*Ulva*				*Laminaria*			
			Formol* titrations		Tryptophane test	Amino-N† in 2 cc. mgms.	Formol titrations		Tryptophane test	Amino-N in 2 cc. mgms.	Formol titrations		Tryptophane test	Amino-N in 2 cc. mgms.
			15 days	30 days			15 days	30 days			15 days	30 days		
Peptone	5	neut.	4.35	15.00	+	1.68	2.80	6.75	+	1.06	2.25	5.65	+	1.14
	.....	neut.	.70	1.00	−	.07	.70	1.00	−	.07	.70	1.00	−	.07
	5	alk.	5.30	18.00	+	1.84	3.05	8.35	+	1.19	2.30	4.15	+	.39
	.....	alk.	.55	.85	−	.00	.55	.85	−	.00	.55	.85	−	.00
Casein	5	neut.	8.70	22.00	+	1.49	6.40	17.70	+	.92	5.95	15.60	+	.98
	.....	neut.	5.55	6.10	−	.48	5.55	6.10	−	.49	5.55	6.10	−	.49
	5	alk.	8.60	23.15	+	1.56	6.25	26.35	+	1.10	5.70	14.50	+	.83
	.....	alk.	5.20	5.70	−	.35	5.20	5.70	−	.35	5.20	5.70	−	.35
Water	5	.....	.40	.95	—	.08	.90	1.65	—	.11	1.05	1.40	—	.09

* Values represent the number of cc. N/50 NaOH to neutralize 10 cc. of substrate.

† The amino-nitrogen in 2 cc. of filtrate from phosphotungstic precipitated protein represents 1.4 cc. of the original substrate.

these in neutral and slightly alkaline solution. This was shown in the formol titrations[1] and in the determination of the nitrogen in the amino acids split off. Albumin was slowly acted upon by *Enteromorpha, Mesogloea,* and *Chondrus.* The first and last also hydrolysed the vegetable protein, legumin, to a slight extent—an action that was not shared by *Mesogloea.*

The action of algal powder on the proteolysis of gelatin and albumin in Mett's tubes.—Two lots of Mett's tubes were made up, one containing coagulated egg-white, and the other, 15 per cent gelatin. In each of a series of flasks containing 50 cc. of distilled water, N/200 NaOH and N/200 HCl respectively, were placed one tube each of egg-white and gelatin. Two grams of the powdered tissue from each of the several forms under investigation were added for enzyme action and the usual percentage of toluene used as an antiseptic. The several series were kept for two months at room temperature. At the end of that time the albumin tubes in the alkaline solution containing the algal powder of *Ulva, Enteromorpha, Chondrus,* and *Agardhiella* showed a slight digestion. The checks in the alkaline solution alone showed swelling. However, although this was indicative of action, it was not definite, since the great length of time the protein was in contact with the complex constituents of the tissue may have been a factor in either causing a slight hydrolysis or a contraction of the albumin. On the other hand, *Laminaria, Ascophyllum, Mesogloea,* and *Ceramium* caused no such action. The gelatin tubes showed no evidences of action even after 60 days.

The effect of proteinases on the hardening of gelatin.—Dox ('10) describes a method for testing the hydrolysis of gelatin which consists in keeping the protein in a liquid state during contact with the material being tested for proteolytic activity, then at the end of a stated period noting whether the gelatin congeals when placed in cold water. This method was used in the following way: Five cc. of 20 per cent gelatin were placed in each of a series of test-tubes, and 5 cc. of the standard

[1] The formaldehyde titrations, as used here, were satisfactory only in a general way, i. e., to show relative rather than exact differences in the amounts of amino acids split off. The differences brought out by the amino-nitrogen determinations are much more exact.

"diffusion-extract," described under "carbohydrases," used for action. The contents of the tubes were made neutral, and acid and alkaline to N/200, as was done in the other proteolytic experiments. Five drops of chloroform-thymol were added as an antiseptic. Checks were set up containing the gelatin together with 5 cc. of boiled "diffusion-extract." The tubes were placed in an incubator at 35° C. for a week, at which time they were removed and cooled in running water. All tubes hardened in a short time, showing that no hydrolysis had taken place.

General results for experiments on proteolysis.—The proteolytic activity, although slow, as was the case with the other enzymes investigated, is definite enough to warrant the statement that proteinases and peptases are very generally present in the algae. When present, such enzymes act best under neutral and alkaline conditions. This last finding is interesting in the light of the existing differences of opinion regarding the relative value of acid and alkaline substrates for vegetable proteinases. It will be recalled that Vines ('97) found that acidity favored the proteinase contained in the leaf pitchers of *Nepenthes,* and in a later paper, he states that peptase (hydrolysing albumoses and peptones to amino acids) always act best under faintly acid conditions. Emmerling ('02), on the other hand, demonstrated that the papain of *Carica papaya* acted more rapidly when the substrate was alkaline. Euler ('12) states in a general way that peptases require a neutral or faintly alkaline substrate, and proteinases (tryptases) an acid one.

Of the proteins employed, solutions of casein and peptone prove the most favorable substrates. Albumin in solution is acted upon slowly, but when employed in the form of Mett's tubes, doubt exists regarding its digestion. Legumin appears to be slowly hydrolysed by *Enteromorpha* and *Chondrus,* but not by *Mesogloea.* Gelatin, either in the liquid state or in the form of Mett's tubes, is not attacked. As groups, the "reds" appear more active in proteolysis than do the "greens," while, as was true for carbohydrases, the "browns" show the least activity.

THE AMIDASES

The tissues from the several algae were tested for their ability to split NH_3 from such amino and amido compounds as urea, acetamid, asparagin, and methyl amine. These compounds were used in 1 per cent concentrations. Series were set up in which 50 cc. of the substrate to be tested were placed in flasks together with 2 grams of the powdered tissue and chloroform-thymol as an antiseptic. Checks were used with the nitrogen compounds alone and with the algal tissue in distilled water. The flasks of duplicate series were kept at room temperature and at 35°C. respectively, for 30 days, at the end of which time Folin's method was employed for the determination of any NH_3 that might have been split off. In the collection of the NH_3, Friedrich's improved gas washing bottles containing 250 cc. of N/50 HCl were used. Air was bubbled through by means of a suction pump for two hours, then 25 cc. portions of the collection acid were removed and titrated against N/50 NaOH, with alizarin red as an indicator. In no case was there any action over that evidenced by the checks.

These results are extremely interesting, in the case of *Ulva* especially. This form, as has been shown, thrives in waters where the organic nitrogen content is high. The question would at once arise whether this increased growth were due to the ability of the *Ulva* to break down the protein molecule and thus obtain an increased supply of nitrogen as NH_3, or whether it were due to the activities of the denitrifying bacteria rendering available a larger assimilable supply. That such bacteria are relatively abundant in sewage-contaminated water has been shown in the review of literature. We can conceive of another factor entering in—that of selective formation of enzymes. It might well be that with plenty of the amino-nitrogen available through the activity of bacteria, no amidases would be formed. The possibility of shedding some light on this point led to the experiments following.

Experiments on amidase formation by Chlamydomonas.—*Chlamydomonas* was grown in pure culture upon two different media; one (with one or two modifications, that used by

Schramm ('14)), containing $(NH_4)_2SO_4$ as a source of nitrogen, the other with nitrogen supplied as peptone and asparagin. These media complete were as follows:

A.		B.	
Agar	10.00 grams	Agar	10.00 grams
$(NH_4)_2SO_4$	.25 grams	Peptone	4.00 grams
$MgSO_4.7\ H_2O$	.10 grams	Asparagin	1.00 grams
K_2HPO_4	.10 grams	$MgSO_4.7\ H_2O$	.10 grams
$FeSO_4$	trace	K_2HPO_4	.10 grams
Glucose	10.00 grams	$FeSO_4$	trace
Distilled H_2O	500.00 cc.	Glucose	10.00 grams
		Distilled H_2O	500.00 cc.

These media, designated "A" and "B," were placed in 125 cc. Erlenmeyers, about 25 cc. to each flask, and the flasks placed horizontally until the agar hardened. A relatively large surface was obtained in this way and the harvesting of the alga later was facilitated. In inoculation, the alga was smeared over the surface of the agar to give an even growth. After a growth of thirty days, it was harvested by scraping from the agar surface with a stiff, platinum needle. The cells were then dehydrated with alcohol, acetone and ether, dried, and ground with an equal weight of fine quartz sand. Flasks were set up in duplicate in Wollf wash bottles, using 1 per cent asparagin as a substrate, with an amount representing .35 grams of sand-free algal powder. Checks were run on both the asparagin and the algal powder alone. One-half the series was taken down at the end of 7 days, the other half at the end of 15 days, and the NH_3 split off determined by the Folin method previously employed. The flasks were kept at a temperature of 35°C. The results are given in table xvi.

TABLE XVI

THE ACTION OF DEHYDRATED CHLAMYDOMONAS CELLS UPON ASPARAGIN

Substrate 50 cc. 1 per cent	Weight algal powder	Nitrogen as NH_3 in 50 cc. substrate mgms.			
		7 days	Net N	15 days	Net N
Asparagin	.35 grams "A"	.36	.16	.45	.21
	.35 grams "B"	1.17	.96	1.84	1.60
	.....	.18	.....	.22	.....
Water	.35 grams "A"	.02	.....	.02	.....
	.35 grams "B"	.03	.....	.04	.....

The amount of nitrogen in the checks is so small as to be well within experimental error. The NH_3 split off by powder

"B" would be almost negligible were the findings not so consistent. There is a definiteness about the increase over the checks that can hardly be ignored. In order to get further evidence on this point, however, another series (table XVII) was set up, using urea and asparagin in 1 per cent concentrations as substrates. The flasks were maintained at a temperature of 35°C. for 30 days.

TABLE XVII

THE ACTION OF DEHYDRATED CHLAMYDOMONAS CELLS UPON ASPARAGIN AND UREA

Substrate	Weight algal powder	Nitrogen as NH_3 split off in 30 days mgms.	Net nitrogen mgms.
Asparagin	.5 grams "A"	1.15	.20
	.5 grams "B"	3.10	2.20
	.....	.65	.....
Urea	.5 grams "A"	1.45	.17
	.5 grams "B"	3.70	2.47
	.....	.98	.....
Water	.5 grams "A"	.30	.....
	.5 grams "B"	.25	.....

In this, as in table XVI, the evidence goes to show that although the desamidization is practically negligible where the alga is grown with $(NH_3)_2SO_4$ as a source of nitrogen, it is definite where the nitrogen is supplied in the amino and amido form. The actual splitting is small in any case.

On the basis of the above, we can simply reason by analogy, and yet this analogy points to the fact that the probable reason for the failure to demonstrate amidase in *Ulva* lies in the failure to form that enzyme. This in turn would indicate that the great growth of *Ulva* in sewage-contaminated waters is probably due to the abundance of desamidizing bacteria which those waters maintain—bacteria which break down the protein molecule with the ultimate setting free of NH_3. Nitrogen, as such, becomes directly available to the plant.

NUCLEASES

The presence of nucleases in the algae has already been reported by Teodoresco ('12), but since he investigated only

one of the forms falling within the scope of this study, experiments were carried on to determine the presence or absence of nucleases in one representative of each group, *Ulva, Ceramium,* and *Ascophyllum.*

One-half per cent nuclein was dissolved in N/10 NaOH, the complete solution of the compound being shown by a drop of phenylphthalein. One hundred cc. of this neutral solution were added to each flask together with 3 grams of air-dried algal powder. Toluene was added as an antiseptic. Checks were set up by adding autoclaved algal powder to the nuclein and also by using nuclein solution alone. The flasks were placed at 35–36°C. for 38 days, at the end of which time the phosphoric acid split off was determined as P_2O_5 by the uranium-acetate method.[1] Five cc. of a sodium acetate solution[2] were added to 25 cc. of the nuclein substrate, this brought to a boil and titrated while hot. Potassium ferrocyanide was used as an indicator—a drop of the titration mixture being removed from time to time and brought into contact with a drop of the indicator on a porcelain plate. The results obtained are given in table XVIII.

TABLE XVIII

THE ACTION OF POWDERED TISSUE FROM CERTAIN ALGAE UPON NUCLEIN

Substrate 100 cc. 1 per cent	Weight algal powder	Free H_3PO_4 as P_2O_5 in 100 cc. in 38 days mgms.	Net amount P_2O_5 in 100 cc. mgms.
Nuclein	3 gms. *Ceramium*	70.00	56.25
	3 gms. *Ceramium* boiled	13.75	.....
	3 gms. *Ulva*	56.70	39.20
	3 gms. *Ulva* boiled	17.50	.....
	3 gms. *Ascophyllum*	17.25	.35
	3 gms. *Ascophyllum* bld.	16.90	.....

These findings substantiate those of Teodoresco ('12) regarding the general presence of nucleases in the algae. The values for *Ceramium* agree very well with those he obtained for the same form, i. e., 56.25 milligrams in 38 days at 35°C., as compared with 76.6 milligrams in 51 days at 22–26°C. *Ulva*

[1] The standard solution of the uranium acetate contained 8.8652 grams of the salt in 250 cc. of water, and each cc. by calculation was equivalent to 5 milligrams of P_2O_5.

[2] The sodium acetate solution contained 25 grams of sodium acetate and 25 cc. of 30 per cent acetic acid in 250 cc.

shows less nuclease activity than does *Ceramium,* while *Ascophyllum,* true to its reputation for inactivity, gives a value so small as to be negligible.

An interesting point is brought out by the use of nuclein—one that proves a check on some of the previous proteinase experiments. Nuclein is composed of nucleic acid bound up with some protein (according to Abderhalden, '11, this is albumin) which must be split off by a proteinase before the nuclein residue is exposed to the attack of the nuclease. That unmistakable nuclease activity was evident, only serves to show again the presence of proteolytic enzymes.

OXIDASES AND CATALASES

Oxidases.—Direct and indirect tests for oxidase action, that is, for the oxidases and the so-called peroxidases, were carried out in all cases with fresh tissue. The general method described by Clark ('10) was employed, using guaiacum, alpha naphthol, and phenylphthalin as reagents. Five grams of the fresh tissue, crushed with an equal weight of fine quartz sand, were extracted for half an hour with 25 cc. of distilled water. The extracting fluid was then filtered off, the tissue residue pressed out, and the filtrate made up to 50 cc. Five cc.-portions were placed in test-tubes, and for the direct test, ten drops of the reagent were added; for the indirect test, this amount plus 1 cc. of fresh 3 per cent hydrogen peroxide. In only two cases was direct oxidization observable—with *Agardhiella* and *Ulva.* With the former, direct action was strong with all three reagents, and when peroxide was added an immediate deepening of the color occurred, showing the presence of peroxidases as well. With *Ulva,* however, both direct and indirect tests were only weakly positive. Atkins ('14), it will be remembered, obtained direct tests with but one of twenty-nine diverse algae investigated and indirect tests with but seven. He thought that reducing substances prevent the demonstration of oxidases in other forms. As brought out in the review of literature, Reed ('15[a]) has since demonstrated indirect oxidation of the alpha naphthol-para-phenylenediamine group of compounds by many of these

forms. In the filamentous forms he showed the presence of oxidases by the formation of colored granules within the cells surrounded by these reagents. Reed concludes that oxidases of specific oxidative ability are very generally present in the algae, and where negative results are obtained, either the necessary specific compound is not present or other factors enter in, such as the destruction of the oxidase equilibrium of the cell upon crushing.

Catalases.—Both fresh and air-dried tissue were used for catalase demonstration. In a preliminary series, the addition of 5 cc. of 3 per cent hydrogen peroxide to about a gram of fresh crushed algal tissue showed evolution of oxygen in all cases except one, that of *Mesogloea.* Later, a series (table XIX)

TABLE XIX

CATALASE ACTIVITY OF CERTAIN ALGAE

Alga	Number cc. O_2 evolved at 21.5°C.		
	2 minutes	5 minutes	10 minutes
Ascophyllum	.3	.9	.9
Laminaria	1.4	2.3	2.3
Mesogloea	0.0	0.0	0.0
Ulva	.2	.4	.5
Agardhiella	3.3	4.6	5.6
Chondrus	.4	2.0	2.5
Rhodymenia	.9	1.4	2.0
Ceramium	3.7	5.9	8.2
Potato leaf tissue	22.6	....	....

was set up in which 1 gram of powder was placed in 125 cc. Erlenmeyer flasks, 10 cc. of 3 per cent hydrogen peroxide added, and the oxygen evolved collected in a gas burette over water. The flask in which the action was taking place was shaken every 15 seconds, and the volume of oxygen evolved read at the end of 2, 5, and 10 minutes. The temperature of the room was practically constant during the experiments and no especial precautions were taken to control the temperature of the flask other than keeping the hands away from it during the action. The results are not meant to be quantitatively exact, but they do give the relative catalase activity of the several forms. In addition, air-dried potato leaf tissue that had been in the laboratory about the same length of time as the algal tissue was tested for comparison.

Catalase, so wide-spread in all plant tissues, is found here in all the forms investigated except *Mesogloea.* The "reds" prove more active than the "browns," and these latter slightly more active than the "greens." No alga is strikingly active, however, when compared with potato leaf tissue. Strangely, *Ulva,* most active in regard to the other enzyme groups, is one of the least so here.

General Discussion and Conclusions

The data obtained in the foregoing investigation serve to show that the number of enzymes in the algae that can be isolated, by standard methods at least, is quite limited. This is especially true of the "browns," in two forms of which, *Ascophyllum,* and the *Fucus* of the earlier study, such action is limited to catalase alone. In this group the demonstrable carbohydrases are restricted to very slowly acting diastases in *Laminaria;* in neither *Ascophyllum* nor *Mesogloea* is there the slightest trace of what might be termed carbohydrate hydrolysis. Moreover, negative results are obtained in these forms for most of the other enzymes sought. *Laminaria* shows lipases and catalases (it was not tested for proteolytic or nuclease activity), and action in *Mesogloea* is restricted to lipases and proteinases, both tryptic and ereptic. On the other hand, very general enzymic activity is demonstrable in the "greens" and the "reds"—diastases, dextrinases, lipases, proteinases (tryptic and ereptic), nuclease, and catalase being isolated from the crushed tissue. Oxidase is shown present in one "red," *Agardhiella,* and in one "green," *Ulva.* Such action, as a whole, appears a little more rapid in the "reds" than in the "greens," but no enzyme stands out as being specific for either a group or an alga within a group.

The carbohydrases demonstrated are restricted in their action to those hydrolysing starch, dextrin, glycogen, and laminarin of the polysaccharides used as substrates, and in *Laminaria,* such action was further limited by a failure to act upon glycogen. In no case, in any member of the three groups was there evidence of disaccharides being attacked. While

this is not so surprising perhaps for sucrose and lactose, it is difficult to understand the failure of enzymic hydrolysis of maltose. The results obtained by Kylin ('13) indicate that both dextrose and fructose are found in algal tissues, and reasoning from results found for plant and animal tissues in general, it seems, as is true in those cases, that in the algae, maltose must be broken down to glucose before assimilation can take place. The failure to isolate this enzyme points to the possible presence of some inhibiting factor, rather than to the non-formation of the ferment.

Lipases, acting very slowly, appear wide-spread in algae, being demonstrable in all the forms used in this study excepting *Ascophyllum.* Along with the fact that fats are very generally found in the algae, these results are significant in that they indicate the importance of the rôle these compounds may play as assimilatory products. It is not thought, as was advanced by Reinke ('76), Hansen ('93), and others, that these fats function as the first products of assimilation, but rather, that they act as storage products of more or less importance.

The algae, in general, show the presence of enzymes capable of hydrolysing certain proteins. Casein and peptone in alkaline and neutral solution prove the most favorable substrates of those tested, although legumin and albumin are also slightly attacked. The "greens" and the "reds" are about equally active in this way, the "browns," as usual, acting more slowly. The fact that both native proteins and peptones were hydrolysed, points to the presence of both tryptic and ereptic enzymes. Still further evidence of the presence of the first of these was the splitting of the protein molecule from nuclein preceding the action of nuclease.

Amidases seem not to be formed by any of these algae. The results obtained with *Chlamydomonas,* from which the amidases were isolated when the alga was grown on a medium containing asparagin and peptone as a source of nitrogen but not when the nitrogen was in the form of ammonium sulphate, indicate that such amidase formation may depend upon the nature of the supply of assimilable organic nitrogen. This has a distinct bearing upon the reason for the increased growth of

Ulva in sewage-contaminated waters. In order to break down the proteins present in the surrounding waters and even those in close contact with the plant itself, it would be necessary for the *Ulva* to secrete an extracellular enzyme, since the large protein molecule is not diffusible into the cell. If so secreted, the enzyme would be quickly dissipated in the large volume of surrounding water. Desamidizing bacteria, on the other hand, have been demonstrated in harbor and shore waters where such algae abound. They can come into much more intimate contact with the protein than can the plant, and undoubtedly play an important rôle in rendering available at all times an abundant supply of organic nitrogen.

The demonstration of nucleases acting upon the previously split nuclein molecule, substantiates the findings of Teodoresco for this enzyme. Both *Ulva* and *Ceramium* showed the presence of the ferment, while *Ascophyllum,* the only representative of the "browns" investigated, gave negative results. Where such enzymes were formed, they compared more favorably with enzymes of fungi and higher plants than do any of the other algal ferments.

None of the "browns" studied showed the presence of oxidative enzymes, while in the "reds" and the "greens" but one form gave the characteristic reactions. It is interesting to note that these algae, *Agardhiella* and *Ulva,* were the most enzymatically active forms studied. The oxidase reactions with guaiacum, alpha naphthol, and phenylphthalin were very positive, both directly, and indirectly with hydrogen peroxide.

In all cases where enzymes were demonstrated, the action was very slow, being with the exception of nuclease, much less rapid than in the higher plants. The reason for this is not clear, but it cannot in all instances be due to inhibiting substances set free upon the death of the cell. Arber ('01), as has been mentioned before, found that *Ulva, Cladophora,* and *Enteromorpha,* placed in the dark but under otherwise presumably normal conditions, required from two weeks with *Ulva,* to two months and more in the case of *Enteromorpha* for destarching. This indicates the presence of a very slowly acting diastase in the cells of these algae. The metabolism

of the algae is also probably slower than that of the higher plants and one might expect, *a priori,* the enzymes also to be less rapid in their action. Although the algal enzymes may be inherently slow, it seems that there may also be substances set free on the death of the cell which either partially or entirely inhibit enzyme action. The writer has found evidence in some preliminary experiments, that the action of taka diastase upon starch is directly proportional to the amount of free tannin present. In connection with this, it was also found that *Ascophyllum* had a "tannoidal" content of 1.1 per cent of the dry weight. It is possible that such tannoids, if in an uncombined state, may after the death of the cell unite with an enzyme to throw it out of the sphere of action. That diastases are demonstrable in tissues having a high tannin content may perhaps be explained on the basis that they are bound up in such a way as to render them incapable of uniting with the ferments. Still other organic inhibiting compounds may be present, and the point opens up a very interesting problem concerning inhibition, not only in algal tissues, but in those of many higher plants as well.

Summary

1. Using standard methods of enzyme isolation and determination, the following enzymes have been found in fresh or dried algal tissue:

a. Carbohydrases hydrolysing the polysaccharides, starch, dextrin, glycogen, and laminarin, but not those hydrolysing the several disaccharides employed as substrates.

b. Lipases acting upon neutral fats but not upon the esters of the lower fatty acids.

c. Proteinases (tryptic and ereptic) acting best under neutral and alkaline conditions.

d. Nucleases.

e. Oxidases and peroxidases (in but two forms—*Agardhiella* and *Ulva*).

f. Catalases.

2. Negative results were obtained for cellulase, cytase, maltase, lactase, sucrase, amidase, and esterase.

3. The action of all the enzymes isolated was very slow.

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GENERAL INDEX

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